(FILE 'HOME' ENTERED AT 11:56:17 ON 30 DEC 2003)

FILE 'CAPLUS, MEDLINE, USPATFULL' ENTERED AT 11:57:44 ON 30 DEC 2003

L1	2815	S	GENISTEIN AND DAIDZEIN
L2	683	S	L1 AND (BENIGN BREAST DISEASE OR CANCER)
L3	262	S	L2 AND PHYTOESTROGEN
L4	95	S	L3 AND BIOCHANIN
L5	54	S	L4 AND FORMONONETIN
L6	19	S	L5 AND PROSTATE CANCER

soy + molasses isoflavones

L6 ANSWER 1 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:327392 CAPLUS
DOCUMENT NUMBER: 136:385486
TITLE: In the prostatic epithelium, dietary isoflavones from red clover significantly increase estrogen receptor .beta. and E-cadherin expression but decrease transforming growth factor .beta.1
AUTHOR(S): Slater, M.; Brown, D.; Husband, A.
CORPORATE SOURCE: Institute for Biomedical Research, The University of Sydney, Australia
SOURCE: Prostate Cancer and Prostatic Diseases (2002), 5(1), 16-21
CODEN: PCPDFW; ISSN: 1365-7852
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In mice fed a diet supplemented with red clover isoflavones the prostatic epithelium displays a significant increase in the prodn. of estrogen receptor .beta. and the adhesion protein E-cadherin but a decrease in transforming growth factor .beta.1. These proteins are estrogenically-induced markers of proliferation, maintenance of histol. architecture, preservation of cell phenotype and redn. of the potential for neoplastic and metastatic transformation. This study suggests that red clover isoflavones represent a non-toxic dietary treatment for prostatic hyperplasia and a redn. in the potential for neoplastic transformation.
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:293254 CAPLUS
DOCUMENT NUMBER: 122:54387
TITLE: Rapid HPLC analysis of dietary **phytoestrogens** from legumes and from human urine
AUTHOR(S): Franke, Adrian A.; Custer, Laurie J.; Cerna, Carmencita M.; Narala, Kavitha
CORPORATE SOURCE: Molecular Carcinogenesis Program, Cancer Research Center Hawaii, Honolulu, HI, 96813, USA
SOURCE: Proceedings of the Society for Experimental Biology and Medicine (1995), 208(1), 18-26
CODEN: PSEBAA; ISSN: 0037-9727
PUBLISHER: Blackwell
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Due to growing evidence suggesting that **phytoestrogens** might protect against various **cancers**, particularly against breast and **prostate cancer**, it is important to measure the exposure of populations to these compds. by detg. levels in food and in human tissue or body fluids to assess the possible **cancer** protective properties of these agents. Therefore, a simple and fast procedure was developed to ext. and simultaneously hydrolyze **phytoestrogens** and their conjugates from food items, and a fast and selective high-performance liq. chromatog. (HPLC) method is presented for precise detns. of the most common dietary **phytoestrogens** - **genistein**, **biochanin**-A, **daidzein**, **formononetin**, and coumestrol - with flavone as internal std. For the first time HPLC was applied to measure these **phytoestrogens** and their most abundant metabolites equol and O-desmethyl-angolensin from human urine. The proposed methodol. has been evaluated for losses due to thermal degrdn. during extn. and hydrolysis and due to sample handling during the entire work-up including solid phase extn., and values are given for inter- and intra-assay variability. Isoflavonoid levels of most common peas and beans used in "western" and "eastern" diets are presented,

and isoflavonoid and coumestrol levels of raw, canned, and cooked foods are compared. Human urinary levels were also detd. with the methodol. comparing values before and after soybean intake.

L6 ANSWER 3 OF 19 MEDLINE on STN
ACCESSION NUMBER: 95199369 MEDLINE
DOCUMENT NUMBER: 95199369 PubMed ID: 7892289
TITLE: Rapid HPLC analysis of dietary **phytoestrogens** from legumes and from human urine.
AUTHOR: Franke A A; Custer L J; Cerna C M; Narala K
CORPORATE SOURCE: Molecular Carcinogenesis Program, Cancer Research Center of Hawaii, Honolulu 96813.
SOURCE: PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, (1995 Jan) 208 (1) 18-26.
Journal code: 7505892. ISSN: 0037-9727.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950427
Last Updated on STN: 19950427
Entered Medline: 19950414

AB Due to growing evidence suggesting that **phytoestrogens** might protect against various **cancers**, particularly against breast and **prostate cancer**, it is important to measure the exposure of populations to these compounds by determining levels in food and in human tissue or body fluids to assess the possible **cancer** protective properties of these agents. Therefore, we developed a simple and fast procedure to extract and simultaneously hydrolyze **phytoestrogens** and their conjugates from food items, and present a fast and selective high-performance liquid chromatography (HPLC) method for precise determinations of the most common dietary **phytoestrogens genistein**, **biochanin**-A, **daidzein**, **formononetin**, and coumestrol using flavone as internal standard. For the first time HPLC was applied to measure these **phytoestrogens** and their most abundant metabolites equol and O-desmethyl-angolensin from human urine. The proposed methodology has been evaluated for losses due to thermal degradation during extraction and hydrolysis and due to sample handling during the entire work-up including solid phase extraction, and values are given for inter- and intra-assay variability. We present isoflavonoid levels of most common peas and beans used in "western" and "eastern" diets and compare isoflavonoid and coumestrol levels of raw, canned, and cooked foods which can be used in future epidemiological studies. We also determined human urinary levels with our methodology comparing values before and after soybean intake.

L6 ANSWER 4 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2003:273233 USPATFULL
TITLE: Method for chemoprevention of **prostate cancer**
INVENTOR(S): Steiner, Mitchell S., Germantown, TN, United States
Raghow, Sharan, Collierville, TN, United States
PATENT ASSIGNEE(S): The University of Tennessee Research Corporation, Knoxville, TN, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6632447	B1	20031014
APPLICATION INFO.:	US 2000-707766		20001108 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2000-531472, filed on 20 Mar 2000, now patented, Pat. No. US 6413533
Continuation-in-part of Ser. No. US 2000-660184, filed on 12 Sep 2000, now patented, Pat. No. US 6413534

Continuation-in-part of Ser. No. US 2000-660191, filed on 12 Sep 2000, now patented, Pat. No. US 6410043 Continuation-in-part of Ser. No. US 2000-660197, filed on 12 Sep 2000, now patented, Pat. No. US 6413535 Continuation-in-part of Ser. No. US 1999-436208, filed on 8 Nov 1999 Continuation-in-part of Ser. No. US 1999-306958, filed on 7 May 1999, now patented, Pat. No. US 6265448

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-84602P	19980507 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Page, Thurman K.
ASSISTANT EXAMINER: Fubara, Blessing
LEGAL REPRESENTATIVE: Eitan, Pearl, Latzer & Cohen Zedek, LLP., Cohen, Mark S.
NUMBER OF CLAIMS: 34
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1342
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to the chemoprevention of **prostate cancer** and, more particularly, to a method of suppressing or inhibiting latent **prostate cancer** comprising administering to a mammalian subject a chemopreventive agent and analogs and metabolites thereof. The chemopreventive agent prevents, prevents recurrence of, suppresses or inhibit prostate carcinogenesis; and treats **prostate cancer**.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 5 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2003:270779 USPATFULL
TITLE: Medical composition for balancing bodily processes
INVENTOR(S): Bland, Jeffrey S., Fox Island, WA, UNITED STATES
Liska, DeAnn J., Tacoma, WA, UNITED STATES
Krumhar, Kim C., Carlsbad, CA, UNITED STATES
Tripp, Matthew, Gig Harbor, WA, UNITED STATES
Darland, Gary K., Gig Harbor, WA, UNITED STATES
Lerman, Robert, Gig Harbor, WA, UNITED STATES
Lukaczer, Daniel O., Gig Harbor, WA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003190381	A1	20031009
APPLICATION INFO.:	US 2003-352388	A1	20030127 (10)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2002-56858, filed on 23 Jan 2002, PENDING

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-265908P	20010202 (60)
	US 2002-352016P	20020125 (60)
	US 2002-432689P	20021211 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 35
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Page(s)
LINE COUNT: 2606

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Medical compositions and methods using same to nutritionally support balance of bodily processes are disclosed. A medical composition to nutritionally support balance of bodily processes involving S-adenosylmethionine is disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 6 OF 19 USPATFULL on STN

ACCESSION NUMBER:	2003:250559 USPATFULL			
TITLE:	Isoflavone composition for oral delivery			
INVENTOR(S):	Hite, Michael P., Seattle, WA, UNITED STATES Turner, Stephen J., Covington, WA, UNITED STATES Federici, Catherine, Seattle, WA, UNITED STATES			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003175345	A1	20030918	
APPLICATION INFO.:	US 2002-313129	A1	20021206	(10)
	NUMBER		DATE	
PRIORITY INFORMATION:	US 2001-339887P		20011206	(60)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	APPLICATION			
LEGAL REPRESENTATIVE:	DAVIS WRIGHT TREMAINE, LLP, 2600 CENTURY SQUARE, 1501 FOURTH AVENUE, SEATTLE, WA, 98101-1688			
NUMBER OF CLAIMS:	49			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	8 Drawing Page(s)			
LINE COUNT:	1038			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A controlled release delivery system composition and method for oral administration of an isoflavone is disclosed

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 7 OF 19 USPATFULL on STN

ACCESSION NUMBER:	2003:188523 USPATFULL			
TITLE:	Method for chemoprevention of **prostate cancer**			
INVENTOR(S):	Steiner, Mitchell S., Germantown, TN, UNITED STATES Raghow, Sharan, Collierville, TN, UNITED STATES			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003130316	A1	20030710	
APPLICATION INFO.:	US 2002-300939	A1	20021121	(10)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 2000-707766, filed on 8 Nov 2000, PENDING Continuation-in-part of Ser. No. US 2000-531472, filed on 20 Mar 2000, GRANTED, Pat. No. US 6413533			
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	APPLICATION			
LEGAL REPRESENTATIVE:	Eitan, Pearl, Latzer & Cohen Zedek, LLP., Suite 1001, 10 Rockefeller Plaza, New York, NY, 10020			
NUMBER OF CLAIMS:	20			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	6 Drawing Page(s)			
LINE COUNT:	1167			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to the chemoprevention of **prostate cancer** and, more particularly, to 1) methods of treating a mammalian subject with **prostate cancer**; 2) methods

of suppressing or inhibiting **prostate cancer** in a mammalian subject; 3) methods of reducing the risk of developing **prostate cancer** in a mammalian subject; 4) methods of treating precancerous precursors of prostate adenocarcinoma lesions in a mammalian subject; 5) methods of suppressing or inhibiting precancerous precursors of prostate adenocarcinoma lesions in a mammalian subject; and 6) methods of reducing the amount of precancerous precursors of prostate adenocarcinoma lesions in a mammalian subject; by administering a chemopreventive agent such as raloxifene, as described herein.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 8 OF 19 USPATFULL on STN

ACCESSION NUMBER:	2003:129703 USPATFULL
TITLE:	Methods for treating or reducing prediposition to breast **cancer**, pre-menstrual syndrome or symptoms associated with menopause by administration of phyto-estrogen
INVENTOR(S):	Kelly, Graham Edmund, Northbridge, AUSTRALIA
PATENT ASSIGNEE(S):	Novogen Research Pty Limited, New South Wales, AUSTRALIA (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6562380	B1	20030513
APPLICATION INFO.:	US 1997-910837		19970813 (8)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 338567, now patented, Pat. No. US 5830887		

	NUMBER	DATE
PRIORITY INFORMATION:	AU 1992-2511	19920519

DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Wilson, James O.
ASSISTANT EXAMINER:	Lewis, Patrick
LEGAL REPRESENTATIVE:	Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
NUMBER OF CLAIMS:	23
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	0 Drawing Figure(s); 0 Drawing Page(s)
LINE COUNT:	1153

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Phyto-estrogen-containing health supplement compositions containing any two or more of **Genistein, Daidzein, Formononetin** and **Biochanin** A, or the natural glycosides thereof are administered for treating or reducing predisposition to breast **cancer**, pre-menstrual syndrome or symptoms of menopause.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 9 OF 19 USPATFULL on STN

ACCESSION NUMBER:	2002:340163 USPATFULL
TITLE:	Dietary supplements comprising soy hypocotyls containing at least one isoflavone
INVENTOR(S):	Kelly, Graham Edmund, Northbridge, AUSTRALIA
PATENT ASSIGNEE(S):	Novogen Research Pty. Ltd., AUSTRALIA (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6497906	B1	20021224
APPLICATION INFO.:	US 2000-547100		20000411 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1997-910837, filed on 13		

Aug 1997 Continuation of Ser. No. US 338567, now patented, Pat. No. US 5830887

	NUMBER	DATE
PRIORITY INFORMATION:	AU 1992-2511	19920519

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Wilson, James O.
LEGAL REPRESENTATIVE: Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
NUMBER OF CLAIMS: 16
EXEMPLARY CLAIM: 1,15
NUMBER OF DRAWINGS: 0 Drawing Figure(s); 0 Drawing Page(s)
LINE COUNT: 1158

AB Composition enriched with natural phyto-estrogens or analogs thereof selected from **Genistein**, **Daidzein**, Formomometin, and **Biochanin** A. These may be used as food additives, tablets, or capsules for promoting health in cases of **cancer**, premenstrual syndrome, menopause, or hypercholestrolaemia.

L6 ANSWER 10 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2002:287201 USPATFULL
TITLE: ENRICHED SPREADS
INVENTOR(S): KIM CHEN, MANDY, BALTIMORE, MD, UNITED STATES
PATRICK, MATTHEW, ANNAPOLIS, MD, UNITED STATES
REDDY, PODUTOORI RAVINDER, COLUMBIA, MD, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002160060	A1	20021031	
APPLICATION INFO.:	US 1999-299778	A1	19990426	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: UNILEVER, PATENT DEPARTMENT, 45 RIVER ROAD, EDGEWATER, NJ, 07020
NUMBER OF CLAIMS: 22
EXEMPLARY CLAIM: 1
LINE COUNT: 774
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB In accordance with a first aspect of the invention, it has now been found that a beneficial form for ingestion of **phytoestrogens** is in the form of a water-in-oil spread. For instance, it has been discovered that **phytoestrogens** can advantageously be consumed, particularly in elevated amounts, when included in the form of a bread spread. It can be expected that the reported beneficial health effects of **phytoestrogens** may be enjoyed by the consumer by consuming the spread without the need for pharmaceutical type pills, capsules, etc. Moreover, the spreads of the invention have good taste, notwithstanding the presence of the often-bitter tasting isoflavones.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 11 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2002:160374 USPATFULL
TITLE: Method for chemoprevention of **prostate cancer**
INVENTOR(S): Steiner, Mitchell S., Germantown, TN, United States
Raghow, Sharan, Collierville, TN, United States
PATENT ASSIGNEE(S): The University of Tennessee Research Corporation, Knoxville, TN, United States (U.S. corporation)

	NUMBER	KIND	DATE

PATENT INFORMATION: US 6413535 B1 20020702
APPLICATION INFO.: US 2000-660197 20000912 (9)
RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2000-531472, filed on 20 Mar 2000 Continuation-in-part of Ser. No. US 1999-436208, filed on 8 Nov 1999 Continuation-in-part of Ser. No. US 1999-306958, filed on 7 May 1999

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-84602P	19980507 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Page, Thurman K.
ASSISTANT EXAMINER: Fubara, Blessing
LEGAL REPRESENTATIVE: Eitan, Pearl, Latzer & Cohen-Zedek, Cohen, Mark S.
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1320
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides the chemoprevention of **prostate cancer** and, more particularly, to a method of preventing prostate carcinogenesis comprising the steps of administering to a human subject having a precancerous precursor of prostate adenocarcinoma, a pharmaceutical preparation comprising a chemopreventive agent to prevent, prevent recurrence of, suppress or inhibit prostate carcinogenesis. The present invention provides a safe and effective method for suppressing or inhibiting latent **prostate cancer** and is particularly useful for treating subjects having elevated risk of developing **prostate cancer**, for example, those having benign prostatic hyperplasia, prostate intraepithelial neoplasia (PIN), or an abnormally high level of circulating prostate specific antibody (PSA), or who have a family history of **prostate cancer**.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 12 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2002:160373 USPATFULL
TITLE: Method for chemoprevention of **prostate cancer**
INVENTOR(S): Steiner, Mitchell S., Germantown, TN, United States
Raghow, Sharan, Collierville, TN, United States
PATENT ASSIGNEE(S): The University of Tennessee Research Corporation, Knoxville, TN, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6413534	B1	20020702
APPLICATION INFO.:	US 2000-660184		20000912 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2000-531472, filed on 20 Mar 2000 Continuation-in-part of Ser. No. US 1999-436208, filed on 8 Nov 1999 Continuation-in-part of Ser. No. US 1999-306958, filed on 7 May 1999, now patented, Pat. No. US 6265448

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-84602P	19980507 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Page, Thurman K.
ASSISTANT EXAMINER: Fubara, Blessing
LEGAL REPRESENTATIVE: Eitan, Pearl, Latzer & Cohen-Zedek, Cohen, Mark S.

NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1278
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides the chemoprevention of **prostate cancer** and, more particularly, to a method of preventing prostate carcinogenesis comprising the steps of administering to a human subject having a precancerous precursor of prostate adenocarcinoma, a pharmaceutical preparation comprising a chemopreventive agent to prevent, prevent recurrence of, suppress or inhibit prostate carcinogenesis. The present invention provides a safe and effective method for suppressing or inhibiting latent **prostate cancer** and is particularly useful for treating subjects having elevated risk of developing **prostate cancer**, for example, those having benign prostatic hyperplasia, prostate intraepithelial neoplasia (PIN), or an abnormally high level of circulating prostate specific antibody (PSA), or who have a family history of **prostate cancer**.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 13 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2002:160372 USPATFULL
TITLE: Method for chemoprevention of **prostate cancer**
INVENTOR(S): Steiner, Mitchell S., Germantown, TN, United States
Raghow, Sharan, Collierville, TN, United States
PATENT ASSIGNEE(S): The University of Tennessee Research Corporation, Knoxville, TN, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6413533	B1	20020702
APPLICATION INFO.:	US 2000-531472		20000320 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1999-436208, filed on 8 Nov 1999 Continuation-in-part of Ser. No. US 1999-306958, filed on 7 May 1999, now patented, Pat. No. US 6265448

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-84602P	19980507 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Page, Thurman K.
ASSISTANT EXAMINER: Fubara, Blessing
LEGAL REPRESENTATIVE: Eitan, Pearl, Latzer & Cohen-Zedek, Cohen, Mark S.
NUMBER OF CLAIMS: 34
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1291
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides the chemoprevention of **prostate cancer** and, more particularly, to a method of preventing prostate carcinogenesis comprising the steps of administering to a human subject having a precancerous precursor of prostate adenocarcinoma, a pharmaceutical preparation comprising a chemopreventive agent to prevent, prevent recurrence of, suppress or inhibit prostate carcinogenesis. The present invention provides a safe and effective method for suppressing or inhibiting latent **prostate cancer** and is particularly useful for treating subjects having elevated risk of developing **prostate cancer**, for example, those having benign prostatic hyperplasia, prostate

intraepithelial neoplasia (PIN), or an abnormally high level of circulating prostate specific antibody (PSA), or who have a family history of **prostate cancer**.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 14 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2002:152226 USPATFULL
TITLE: Method for chemoprevention of **prostate cancer**
INVENTOR(S): Steiner, Mitchell S., Germantown, TN, United States
Raghow, Sharan, Collierville, TN, United States
PATENT ASSIGNEE(S): The University of Tennessee Research Corporation, Knoxville, TN, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6410043	B1	20020625
APPLICATION INFO.:	US 2000-660191		20000912 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2000-531472, filed on 20 Mar 2000 Continuation-in-part of Ser. No. US 1999-436208, filed on 8 Nov 1999 Continuation-in-part of Ser. No. US 1999-306958, filed on 7 May 1999

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-84602P	19980507 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Page, Thurman K.
ASSISTANT EXAMINER: Fubara, Blessing
LEGAL REPRESENTATIVE: Eitan, Pearl, Latzer & Cohen-Zedek, Cohen, Mark S.
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1268
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides the chemoprevention of **prostate cancer** and, more particularly, to a method of preventing prostate carcinogenesis comprising the steps of administering to a human subject having a precancerous precursor of prostate adenocarcinoma, a pharmaceutical preparation comprising a chemopreventive agent to prevent, prevent recurrence of, suppress or inhibit prostate carcinogenesis. The present invention provides a safe and effective method for suppressing or inhibiting latent **prostate cancer** and is particularly useful for treating subjects having elevated risk of developing **prostate cancer**, for example, those having benign prostatic hyperplasia, prostate intraepithelial neoplasia (PIN), or an abnormally high level of circulating prostate specific antibody (PSA), or who have a family history of **prostate cancer**.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 15 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2002:129533 USPATFULL
TITLE: Method of preparing and using isoflavones for the treatment of alcoholism
INVENTOR(S): Empie, Mark, Forsyth, IL, United States
Gugger, Eric, Latham, IL, United States
PATENT ASSIGNEE(S): Archer Daniels Midland Company, Decatur, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE

PATENT INFORMATION:	US 6399072	B1	20020604
APPLICATION INFO.:	US 2000-615152		20000713 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1998-162038, filed on 28 Sep 1998, now patented, Pat. No. US 6261565
Continuation-in-part of Ser. No. US 1998-35588, filed on 5 Mar 1998, now patented, Pat. No. US 6033714
Continuation-in-part of Ser. No. US 1997-868629, filed on 4 Jun 1997, now patented, Pat. No. US 5792503
Division of Ser. No. US 1996-614545, filed on 13 Mar 1996, now patented, Pat. No. US 5702752

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-60549P	19971002 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Gitomer, Ralph
ASSISTANT EXAMINER: Khare, Devesh
LEGAL REPRESENTATIVE: Michael Best & Friedrich LLC, Whitesel, J. Warren
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 0 Drawing Figure(s); 0 Drawing Page(s)
LINE COUNT: 580
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A composition is prepared by extracting phytochemicals from plant matter for treatment of alcohol dependency. This composition is enriched preferably with two or more fractions of plant matter, namely: isoflavones, lignans, saponins, sapogenins, catechins and phenolic acids. Soy is the preferred source of these chemicals; however, other plants may also be used, such as wheat, psyllium, rice, oats, red clover, kudzu, alfalfa, flax, and cocoa. The composition is a dietary supplement for treatment of alcoholism. The isoflavone may be any in a group including malonyl, acetyl, glucoside, and aglycone. The composition is in a concentrated form to be delivered in an easy to consume dosage, such as a pill, tablet, liquid, capsule, or a food supplement including a health bars.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 16 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2002:115789 USPATFULL
TITLE: Method of preparing and using isoflavones for the treatment of blood related illnesses
INVENTOR(S): Empie, Mark, Forsyth, IL, United States
Gugger, Eric, Latham, IL, United States
PATENT ASSIGNEE(S): Archer Daniels Midland Company, Decatur, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6391308	B1	20020521
APPLICATION INFO.:	US 2000-615239		20000713 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1998-162038, filed on 28 Sep 1998, now patented, Pat. No. US 6261565
Continuation-in-part of Ser. No. US 1998-35588, filed on 5 Mar 1998, now patented, Pat. No. US 6033714
Continuation-in-part of Ser. No. US 1997-868629, filed on 4 Jun 1997, now patented, Pat. No. US 5792503
Division of Ser. No. US 1996-614545, filed on 13 Mar 1996, now patented, Pat. No. US 5702752

	NUMBER	DATE

PRIORITY INFORMATION: US 1997-60549P 19971002 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Gitomer, Ralph
ASSISTANT EXAMINER: Khare, Devesh
LEGAL REPRESENTATIVE: Michael Best & Friedrich LLC, Whitesel, J. Warren
NUMBER OF CLAIMS: 16
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 0 Drawing Figure(s); 0 Drawing Page(s)
LINE COUNT: 607
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A composition is prepared by extracting phytochemicals from plant matter and is administered to provide treatment for cardiovascular medical conditions, such as: excessive bloodstream cholesterol, the risk of heart disease, abnormal blood lipid profiles, and abnormal vascular effects. This composition is enriched preferably with two or more fractions of plant matter, namely: isoflavones, lignans, saponins, sapogenins, catechins and phenolic acids. The isoflavones are selected from a group including malonyl, acetyl, glucoside and aglycone. Soy is the preferred source of these chemicals; however, other plants may also be used, such as wheat, psyllium, rice, oats, red clover, kudzu, alfalfa, flax, and cocoa. The composition is a dietary supplement in a concentrated form, preferably in an easy to consume form, for treatment of various cardiovascular conditions and various other related disorders.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 17 OF 19 USPATFULL on STN
ACCESSION NUMBER: 2001:111836 USPATFULL
TITLE: Method of preparing and using isoflavones
INVENTOR(S): Empie, Mark, Forsyth, IL, United States
Gugger, Eric, Latham, IL, United States
PATENT ASSIGNEE(S): Archer Daniels Midland Company, Decatur, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6261565	B1	20010717
APPLICATION INFO.:	US 1998-162038		19980928 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1998-35588, filed on 5 Mar 1998, now patented, Pat. No. US 6033714
Continuation-in-part of Ser. No. US 1997-868629, filed on 4 Jun 1997, now patented, Pat. No. US 5792503
Division of Ser. No. US 1996-614545, filed on 13 Mar 1996, now patented, Pat. No. US 5702752

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-60549P	19971002 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Gitomer, Ralph
ASSISTANT EXAMINER: Khare, D
LEGAL REPRESENTATIVE: Laff, Whitesel & Saret, Ltd., Whitesel, J. Warren
NUMBER OF CLAIMS: 54
EXEMPLARY CLAIM: 1
LINE COUNT: 762
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides for a refinement of phytochemicals in order to tailor the refined end product to particular human dietary needs. More particularly, a composition is prepared by extracting phytochemicals from plant matter. This composition is enriched preferably in two or more isoflavones, lignans, saponins, catechins and phenolic acids. Soy

is the preferred source of these chemicals; however, other plants may also be used, such as red clover, kudzu, flax, and cocoa. The composition is a dietary supplement for treatment of various **cancers**, pre-and-post-menstrual syndromes, and various other disorders.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 18 OF 19 USPATFULL on STN

ACCESSION NUMBER:	2000:137885 USPATFULL		
TITLE:	Vegetable protein composition containing an isoflavone depleted vegetable protein material with an isoflavone containing material		
INVENTOR(S):	Holbrook, James L., Troy, IL, United States Waggle, Doyle H., St. Louis, MO, United States		
PATENT ASSIGNEE(S):	Protein Technologies International, Inc., St. Louis, MO, United States (U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6132795		20001017
APPLICATION INFO.:	US 1998-44961		19980315 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Weier, Anthony J.		
LEGAL REPRESENTATIVE:	Taylor, Richard B.		
NUMBER OF CLAIMS:	57		
EXEMPLARY CLAIM:	1		
LINE COUNT:	1290		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is a vegetable protein composition. The vegetable protein composition contains an isoflavone depleted vegetable protein material and an isoflavone containing material which is dispersed in the isoflavone depleted vegetable protein material. The composition may be used in foods to provide the nutritional benefits of the isoflavone depleted vegetable protein material while providing the health benefits of the isoflavone containing material. The invention also includes processes for forming such vegetable protein compositions.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 19 OF 19 USPATFULL on STN

ACCESSION NUMBER:	1998:135034 USPATFULL		
TITLE:	Health supplements containing phyto-oestrogens, analogues or metabolites thereof		
INVENTOR(S):	Kelly, Graham Edmund, Northbridge, Australia		
PATENT ASSIGNEE(S):	Novogen Research Pty. Ltd., New South Wales, Australia (non-U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5830887		19981103
	WO 9323069		19931125
APPLICATION INFO.:	US 1995-338567		19950112 (8)
	WO 1993-AU230		19930519
			19950112 PCT 371 date
			19950112 PCT 102(e) date
	NUMBER		DATE
PRIORITY INFORMATION:	AU 1992-2511		19920519
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Kunz, Gary L.		

LEGAL REPRESENTATIVE: Dann, Dorfman, Herrell and Skillman
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
LINE COUNT: 818
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compositions enriched with natural phyto-oestrogens or analogues thereof selected from **Genistein**, **Daidzein**, **Formononetin** and **Biochanin** A. These may be used as food additives, tablets or capsules for promoting health in cases of **cancer**, pre-menstrual syndrome, menopause or hypercholesterolaemia.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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L3 ANSWER 412 OF 540 MEDLINE on STN
ACCESSION NUMBER: 93072693 MEDLINE
DOCUMENT NUMBER: 93072693 PubMed ID: 1443418
TITLE: Isolation and identification of **phytoestrogens** from beer.
AUTHOR: Rosenblum E R; Campbell I M; Van Thiel D H; Gavaler J S
CORPORATE SOURCE: Department of Medicine, University of Pittsburgh School of Medicine, PA.
SOURCE: ALCOHOLISM, CLINICAL AND EXPERIMENTAL RESEARCH, (1992 Oct) 16 (5) 843-5.
Journal code: 7707242. ISSN: 0145-6008.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19930122
Entered Medline: 19921208

TI Isolation and identification of **phytoestrogens** from beer.

AB Two estrogenic substances of plant origin have been identified in beer using gas chromatography/mass spectrometry. These **phytoestrogens**, **daidzein** and **genistein**, have previously been shown to be biologically active in animals. Confirming the presence of biologically active **phytoestrogens** in beer and their possible presence in other beverages, suggests that there may be clinically significant effects related to sustained exposure to **phytoestrogens** contained in alcoholic beverages.

CT Check Tags: Human
*Beer: AN, analysis
*Estrogens: IP, isolation & purification
Estrogens, Non-Steroidal: IP, isolation & purification
Isoflavones: IP, isolation & purification
Mass Fragmentography

RN **486-66-8 (daidzein)**

CN 0 (Estrogens); 0 (Estrogens, Non-Steroidal); 0 (**Isoflavones**); 0 (**phytoestrogens**)

(FILE 'HOME' ENTERED AT 12:55:19 ON 30 DEC 2003)

FILE 'CAPLUS, MEDLINE, USPATFULL' ENTERED AT 12:55:33 ON 30 DEC 2003

L1 9007 S ISOFLAVONE
L2 1190 S L1 AND PHYTOESTROGEN
L3 540 S L2 AND (DAIDZEIN AND GENISTEIN)

=>

L3 ANSWER 399 OF 540 MEDLINE on STN
ACCESSION NUMBER: 95384979 MEDLINE
DOCUMENT NUMBER: 95384979 PubMed ID: 7656220
TITLE: Genetic damage and the inhibition of 7,12-dimethylbenz[a]anthracene-induced genetic damage by the **phytoestrogens, genistein** and **daidzein**, in female ICR mice.
AUTHOR: Giri A K; Lu L J
CORPORATE SOURCE: Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston 77555-1110, USA.
CONTRACT NUMBER: CA56273 (NCI)
MO1-RR00073 (NCRR)
SOURCE: CANCER LETTERS, (1995 Aug 16) 95 (1-2) 125-33.
Journal code: 7600053. ISSN: 0304-3835.
PUB. COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951013
Last Updated on STN: 19980206
Entered Medline: 19951004

TI Genetic damage and the inhibition of 7,12-dimethylbenz[a]anthracene-induced genetic damage by the **phytoestrogens, genistein** and **daidzein**, in female ICR mice.

AB . . . reduced rates of breast, colon and prostate cancer possibly due, in part, to the presence in soybeans of two estrogenic **isoflavones**, **genistein** and **daidzein**. This study investigated the genotoxicity of these soya **isoflavones** and their interactions with 7,12-dimethylbenz[a]anthracene (DMBA)-induced sister chromatid exchanges (SCE) in bone marrow cells and DNA adduct formations in liver and mammary glands of mice. Groups of female ICR mice were pretreated i.p. with **daidzein** and/or **genistein** (10-20 mg/kg per day for 6 days or 50 mg/kg per 12 h for 3 days) or with the solvent,. . . in the DMSO treated controls (P = 0.001). DMBA induced 20% fewer SCE (P < 0.05) in mice pretreated with **daidzein, genistein** or a combination of **genistein** and **daidzein** (6 x 20 mg/kg per day for 6 days) when compared to mice that received no pretreatments. **Genistein** at 50 mg/kg per 12 h for 3 days also inhibited DMBA-induced SCE by 20%. However, treatment for 3 days with 50 mg/kg per 12 h of **genistein** or **daidzein** alone, or a combination of **daidzein** plus **genistein** (without DMBA treatment) also induced more SCE than treatment with only the solvent (DMSO, P < 0.05). Pretreatment with both the low and the high doses of **daidzein** plus **genistein** or the high dose of **genistein** reduced the replication index of bone marrow cells when compared to pretreatment with DMSO (P < 0.05). Pretreatment with **genistein** reduced DMBA-induced DNA adduct formation by 34%, but this was only marginally significant (P = 0.08) due to the large inter-individual variability in adduct levels. These results show that **genistein** and **daidzein** suppress SCE and possibly DNA adduct formation induced by the known carcinogen, DMBA. This response to a low dose **isoflavone** exposure may be partly responsible for the protective effect against endocrine cancers of soya consumption.

CT . . . Animal; Female; Support, U.S. Gov't, P.H.S.
*9,10-Dimethyl-1,2-benzanthracene: AI, antagonists & inhibitors
DNA Adducts: CH, chemistry
*DNA Damage: DE, drug effects
Genistein
***Isoflavones: PD, pharmacology**
Mice
Mice, Inbred ICR
Sister Chromatid Exchange: DE, drug effects

RN **446-72-0 (Genistein); 486-66-8 (daidzein);** 57-97-6 (9,10-Dimethyl-1,2-benzanthracene)
CN 0 (DNA Adducts); 0 (**Isoflavones**)

L3 ANSWER 400 OF 540 MEDLINE on STN
ACCESSION NUMBER: 95382551 MEDLINE
DOCUMENT NUMBER: 95382551 PubMed ID: 7653993
TITLE: Structural requirements for differentiation-induction and growth-inhibition of mouse erythroleukemia cells by **isoflavones**.
AUTHOR: Jing Y; Waxman S
CORPORATE SOURCE: Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029, USA.
CONTRACT NUMBER: CA 59936 (NCI)
SOURCE: ANTICANCER RESEARCH, (1995 Jul-Aug) 15 (4) 1147-52. Journal code: 8102988. ISSN: 0250-7005.
PUB. COUNTRY: Greece
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19951005
Last Updated on STN: 20000303
Entered Medline: 19950928

TI Structural requirements for differentiation-induction and growth-inhibition of mouse erythroleukemia cells by **isoflavones**.

AB **Isoflavones** are natural plant **phytoestrogens** which have been shown to have anticancer proliferation, differentiation and chemopreventive effects. In order to determine structure-function requirements, we compared the effects of several **isoflavone** derivatives and one flavone on mouse erythroleukemia (MEL) cell growth and differentiation. All chemicals tested are closely related in structure to **genistein** (4',5,7-trihydroxyisoflavone), a known differentiation inducer and tyrosine protein kinase inhibitor. **Genistein**, **daidzein** (4',7-dihydroxyisoflavone) and genistin (7-glucoside of **genistein**) induced differentiation of MEL cells based on benzidine staining. Biochanin A (5,7-dihydroxy-4'-metho-xyisoflavone) and apigenin (4',5,7-trihydroxyflavone) had no differentiation inducing effect. The potency of these chemicals on cell growth inhibition was apigenin > **genistein** > genistin > biochanin A > **daidzein**. These results suggest that the **isoflavone** structure and 4'-hydroxyl group are essential for the differentiation induction effect, whereas trihydroxyl derivatives are good growth inhibitors. **Daidzein** is a potent differentiation inducer with the least cytotoxic effect.

CT . . .
Agents, Phytogenic: PD, pharmacology
Cell Differentiation: DE, drug effects
Cell Division: DE, drug effects
Chamomile
DNA Damage
Flavones: PD, pharmacology
Genistein
***Isoflavones: PD, pharmacology**
Leukemia, Erythroblastic, Acute: PA, pathology
Mice
Oils, Volatile: PD, pharmacology
Plants, Medicinal
Structure-Activity Relationship
Tumor Cells, Cultured
RN **446-72-0 (Genistein)**
CN 0 (Antineoplastic Agents, Phytogenic); 0 (Flavones); 0 (**Isoflavones**); 0 (Oils, Volatile)

=> d l3 410-450 ibib kwic

L3 ANSWER 410 OF 540 MEDLINE on STN
ACCESSION NUMBER: 93318095 MEDLINE
DOCUMENT NUMBER: 93318095 PubMed ID: 8392221
TITLE: Quantitative determination of lignans and isoflavonoids in plasma of omnivorous and vegetarian women by isotope dilution gas chromatography-mass spectrometry.
AUTHOR: Adlercreutz H; Fotsis T; Lampe J; Wahala K; Makela T; Brunow G; Hase T
CORPORATE SOURCE: Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, Finland.
CONTRACT NUMBER: 1 R01 CA56289-01 (NCI)
SOURCE: SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION. SUPPLEMENT, (1993) 215 5-18.
Journal code: 2984789R. ISSN: 0085-591X.
PUB. COUNTRY: Norway
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199308
ENTRY DATE: Entered STN: 19930820
Last Updated on STN: 19930820
Entered Medline: 19930806

AB The first quantitative method for the determination of both lignans and isoflavonoid **phytoestrogens** in plasma is presented. Using ion-exchange chromatography the diphenols are separated into two fractions 1) the biologically "active" fraction containing. . . estrogen conjugates during the first steps and later by adding deuterated internal standards of all compounds measured (matairesinol, enterodiol, enterolactone, **daidzein**, O-desmethylangolensin, equol, and **genistein**). The final determination is carried out by isotope dilution gas chromatography-mass spectrometry in the selected ion monitoring mode (GC/MS/SIM). The. . . vegetarian women are presented for the first time. The most important findings are that the free+sulfate fraction is low for **genistein** (3.8% of total), but as much as 21-25% of enterolactone and enterodiol occurs in this fraction. A good correlation between. . .

CT . . . Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
*Diet, Vegetarian
*Estrogens: BL, blood
Estrogens: UR, urine
Finland
*Indicator Dilution Techniques
***Isoflavones: BL, blood**
Isoflavones: UR, urine
Lignans
*Lignin: AN, analysis
*Mass Fragmentography
Mass Fragmentography: SN, statistics & numerical data
Menopause
Plants

CN 0 (Estrogens); 0 (**Isoflavones**); 0 (Lignans); 0 (**phytoestrogens**)

L3 ANSWER 411 OF 540 MEDLINE on STN
ACCESSION NUMBER: 93271051 MEDLINE
DOCUMENT NUMBER: 93271051 PubMed ID: 8499347
TITLE: In vitro bioassays of non-steroidal **phytoestrogens**.
AUTHOR: Markiewicz L; Garey J; Adlercreutz H; Gurpide E
CORPORATE SOURCE: Department of Obstetrics, Gynecology & Reproductive Science, Mount Sinai School of Medicine, New York, NY 10029-6574.

SOURCE: JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (1993 May) 45 (5) 399-405.
Journal code: 9015483. ISSN: 0960-0760.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930716
Last Updated on STN: 19980206
Entered Medline: 19930625

TI In vitro bioassays of non-steroidal **phytoestrogens**.

AB . . . of isoflavonoids using human endometrial cells and tissue. The relative estrogenic potencies (EC50 values) of estradiol, 3 dietary isoflavonoids (coumestrol, **genistein** and **daidzein**) and one of their metabolites (equol), were estimated by using a recently developed multiwell plate in vitro bioassay based on. . .

CT . . .
Dinoprost: ME, metabolism
Endometrial Neoplasms
Endometrium: DE, drug effects
Endometrium: ME, metabolism
Estradiol: AA, analogs & derivatives
Estradiol: PD, pharmacology
Genistein
***Isoflavones: PD, pharmacology**
Tamoxifen: AA, analogs & derivatives
Tamoxifen: PD, pharmacology
Tumor Cells, Cultured

RN 10540-29-1 (Tamoxifen); **446-72-0 (Genistein)**; 479-13-0 (Coumestrol); **486-66-8 (daidzein)**; 50-28-2 (Estradiol); 531-95-3 (equol); 551-11-1 (Dinoprost); 68392-35-8 (4-hydroxytamoxifen); 98007-99-9 (ICI 164384)

CN 0 (Chromans); 0 (**Isoflavones**); EC 3.1.3.1 (Alkaline Phosphatase)

L3 ANSWER 412 OF 540 MEDLINE on STN
ACCESSION NUMBER: 93072693 MEDLINE
DOCUMENT NUMBER: 93072693 PubMed ID: 1443418
TITLE: Isolation and identification of **phytoestrogens** from beer.
AUTHOR: Rosenblum E R; Campbell I M; Van Thiel D H; Gavaler J S
CORPORATE SOURCE: Department of Medicine, University of Pittsburgh School of Medicine, PA.
SOURCE: ALCOHOLISM, CLINICAL AND EXPERIMENTAL RESEARCH, (1992 Oct) 16 (5) 843-5.
Journal code: 7707242. ISSN: 0145-6008.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19930122
Entered Medline: 19921208

TI Isolation and identification of **phytoestrogens** from beer.

AB Two estrogenic substances of plant origin have been identified in beer using gas chromatography/mass spectrometry. These **phytoestrogens**, **daidzein** and **genistein**, have previously been shown to be biologically active in animals. Confirming the presence of biologically active **phytoestrogens** in beer and their possible presence in other beverages, suggests that there may be clinically significant effects related to sustained exposure to **phytoestrogens** contained in alcoholic beverages.

CT Check Tags: Human

*Beer: AN, analysis
*Estrogens: IP, isolation & purification
Estrogens, Non-Steroidal: IP, isolation & purification
Isoflavones: IP, isolation & purification
Mass Fragmentography
RN **486-66-8 (daidzein)**
CN 0 (Estrogens); 0 (Estrogens, Non-Steroidal); 0 (**Isoflavones**); 0 (**phytoestrogens**)

L3 ANSWER 413 OF 540 MEDLINE on STN
ACCESSION NUMBER: 91182616 MEDLINE
DOCUMENT NUMBER: 91182616 PubMed ID: 2009221
TITLE: The estrogenic activity of certain **phytoestrogens** in the Siberian sturgeon Acipenser baeri.
AUTHOR: Pelissero C; Bennetau B; Babin P; Le Menn F; Dunogues J
CORPORATE SOURCE: Laboratoire de Biologie Marine, Universite de Bordeaux I, Talence, France.
SOURCE: JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (1991 Mar) 38 (3) 293-9.
Journal code: 9015483. ISSN: 0960-0760.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910526
Last Updated on STN: 19980206
Entered Medline: 19910507
TI The estrogenic activity of certain **phytoestrogens** in the Siberian sturgeon Acipenser baeri.
AB Various **phytoestrogens** such as formononetin, **daidzein**, **genistein** and equol were synthesized. Their purity was assessed by various analytical techniques including melting point determination, thin-layer chromatography (TLC), infra-red. . . biologically tested by the induction of vitellogenin secretion in yearling sturgeon and compared to the activity of estradiol-17 beta. Pure **daidzein**, biochanin A, **genistein**, equol and coumestrol all had estrogenic activity as assessed by their induction of hepatic synthesis of vitellogenin when administrated intraperitoneally. . . the most vitellogenin secretion with the lowest dose administered. Formononetin was inactive when administered by the intraperitoneal route. All the **phytoestrogens** tested were considerably less potent than estradiol-17 beta.
CT Check Tags: Animal
Chromans: CH, chemistry
Chromatography, Thin Layer
Coumestrol: CH, chemistry
*Estrogens: CH, chemistry
*Estrogens: ME, metabolism
Fishes
Genistein
Isoflavones: CH, chemistry
Liver: ME, metabolism
Magnetic Resonance Spectroscopy
Mass Fragmentography
Spectrophotometry, Infrared
Vitellogenin: AN, analysis
Vitellogenin: BI, biosynthesis
RN **446-72-0 (Genistein)**; 479-13-0 (Coumestrol); **486-66-8 (daidzein)**; 491-80-5 (biochanin A); 531-95-3 (equol)
CN 0 (Chromans); 0 (Estrogens); 0 (**Isoflavones**); 0 (Vitellogenin); 0 (**phytoestrogens**)

L3 ANSWER 414 OF 540 MEDLINE on STN
ACCESSION NUMBER: 90219838 MEDLINE

DOCUMENT NUMBER: 90219838 PubMed ID: 2139153
TITLE: Interaction of **phytoestrogens** and other environmental estrogens with prostaglandin synthase in vitro.
AUTHOR: Degen G H
CORPORATE SOURCE: Institute of Toxicology, University of Wurzburg, F.R.G.
SOURCE: JOURNAL OF STEROID BIOCHEMISTRY, (1990 Mar) 35 (3-4) 473-9.
Journal code: 0260125. ISSN: 0022-4731.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199005
ENTRY DATE: Entered STN: 19900622
Last Updated on STN: 19900622
Entered Medline: 19900524

TI Interaction of **phytoestrogens** and other environmental estrogens with prostaglandin synthase in vitro.

AB The **phytoestrogens daidzein, genistein,** equol and coumestrol were found to stimulate microsomal prostaglandin H synthase (PHS) in vitro in a concentration-dependent manner when PHS-activity. . . was partially reversed at high concentrations probably due to their antioxidant properties causing inhibition. In contrast, the monomethyl ethers of **daidzein** and **genistein**, formononetin and biochanin A, had little or weakly inhibitory effect on PHS, and appear to be no or poor co-substrates. . .

CT Check Tags: Support, Non-U.S. Gov't
Cyclooxygenase Inhibitors
Equilin: PD, pharmacology
*Estrogens: PD, pharmacology
Indenes: PD, pharmacology
Isoflavones: PD, pharmacology
*Prostaglandin-Endoperoxide Synthase: AN, analysis
Structure-Activity Relationship
Zearalenone: PD, pharmacology

RN 17924-92-4 (Zearalenone); 24643-97-8 (indenestrol); 38028-27-2 (indenestrol B); 474-86-2 (Equilin); **486-66-8 (daidzein)**

CN 0 (Cyclooxygenase Inhibitors); 0 (Estrogens); 0 (Indenes); 0 (**Isoflavones**); 0 (**phytoestrogens**); EC 1.14.99.1 (Prostaglandin-Endoperoxide Synthase)

L3 ANSWER 415 OF 540 MEDLINE on STN
ACCESSION NUMBER: 90149300 MEDLINE
DOCUMENT NUMBER: 90149300 PubMed ID: 2620158
TITLE: The use of thermospray liquid chromatography/tandem mass spectrometry for the class identification and structural verification of **phytoestrogens** in soy protein preparations.
AUTHOR: Barbuch R J; Coutant J E; Welsh M B; Setchell K D
CORPORATE SOURCE: Merrell Dow Research Institute, Cincinnati, Ohio 45215.
SOURCE: BIOMEDICAL AND ENVIRONMENTAL MASS SPECTROMETRY, (1989 Nov) 18 (11) 973-7.
Journal code: 8603224. ISSN: 0887-6134.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19980206
Entered Medline: 19900322

TI The use of thermospray liquid chromatography/tandem mass spectrometry for the class identification and structural verification of **phytoestrogens** in soy protein preparations.

AB The thermospray mass spectra of the **phytoestrogens** have intense protonated molecular ions but contain few or no ions indicative of structure. Tandem mass spectrometry (MS/MS) was used to obtain daughter ion spectra containing ions unique to the different structural characteristics of each **phytoestrogen** subclass and was used both to confirm identification and propose structures for unknowns. In addition to unique daughter ion spectra, MS/MS was used as a class identifier to detect **phytoestrogens** through the neutral loss of 56 (due to consecutive losses of CO) that is common to all members of this family. Several sources of soy protein were investigated to confirm the presence or absence of **phytoestrogens**. In one preparation investigated, **daidzein** and **genistein** were detected as well as an unknown **phytoestrogen** of the Biochanin A subclass. This unknown has been tentatively identified as 6,7-dihydroxy-4'-methoxyisoflavone using its daughter ion spectrum.

CT Animal Feed: AN, analysis
Chemistry
Chromatography, High Pressure Liquid
Chromatography, Liquid
*Estrogens: AN, analysis
Genistein
Indicators and Reagents
Isoflavones: AN, analysis
Soybean Proteins
Spectrophotometry, Ultraviolet
Spectrum Analysis, Mass
*Vegetable Proteins: AN, analysis

RN **446-72-0 (Genistein); 486-66-8 (daidzein)**

CN 0 (Estrogens); 0 (Indicators and Reagents); 0 (**Isoflavones**); 0 (Soybean Proteins); 0 (Vegetable Proteins); 0 (**phytoestrogens**)

L3 ANSWER 416 OF 540 MEDLINE on STN

ACCESSION NUMBER: 89069598 MEDLINE

DOCUMENT NUMBER: 89069598 PubMed ID: 2462136

TITLE: Identification of **phytoestrogens** in the urine of male dogs.

AUTHOR: Juniewicz P E; Pallante Morell S; Moser A; Ewing L L

CORPORATE SOURCE: Department of Population Dynamics, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD 21205.

CONTRACT NUMBER: 2-P30 HD06208 (NICHD)
AM19300 (NIADDK)
HD07204 (NICHD)
+

SOURCE: JOURNAL OF STEROID BIOCHEMISTRY, (1988 Dec) 31 (6) 987-94.
Journal code: 0260125. ISSN: 0022-4731.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198901

ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19980206
Entered Medline: 19890125

TI Identification of **phytoestrogens** in the urine of male dogs.

AB . . . any of their primary metabolites was observed in the present study. We used thermospray-mass spectrometry and GC-MS to identify the **phytoestrogens daidzein**, equol, formononetin and **genistein** in HPLC purified fractions of urine obtained from male beagles. Using the same techniques we also confirmed the presence of **daidzein** and **genistein** in the commercial diet fed to these same dogs. Using the immature rat uterine cytosol estrogen receptor assay, relative binding affinities of 0.08, 1.1, less than 0.01 and 3.9% were obtained for **daidzein**, equol, formononetin and **genistein**, respectively when compared to estradiol (100%). In

conclusion, **phytoestrogens** are present in urine of male beagles. Moreover, the commercial diet fed to these dogs contains **isoflavones** which can be converted to equol by intestinal microflora. These results suggest the need for investigations of **phytoestrogens** (e.g. equol) excreted into the urine daily and its relationship to the incidence and severity of BPH in the dog.

CT . . .

Competitive
Chromans: ME, metabolism
Chromans: UR, urine
Chromatography, High Pressure Liquid
*Dogs: UR, urine
Estrogens: ME, metabolism
*Estrogens: UR, urine
Genistein
Isoflavones: ME, metabolism
Isoflavones: UR, urine
Mass Fragmentography
Prostatic Hyperplasia: ET, etiology
Receptors, Estrogen: ME, metabolism
Reference Values

RN **446-72-0 (Genistein)**; 485-72-3 (formononetin); **486-66-8 (daidzein)**; 531-95-3 (equol)

CN 0 (Chromans); 0 (Estrogens); 0 (**Isoflavones**); 0 (Receptors, Estrogen); 0 (**phytoestrogens**)

L3 ANSWER 417 OF 540 MEDLINE on STN
ACCESSION NUMBER: 81074785 MEDLINE
DOCUMENT NUMBER: 81074785 PubMed ID: 6449666
TITLE: Lack of mutagenicity of some **phytoestrogens** in the salmonella/mammalian microsome assay.
AUTHOR: Bartholomew R M; Ryan D S
SOURCE: MUTATION RESEARCH, (1980 Aug) 78 (4) 317-21. Journal code: 0400763. ISSN: 0027-5107.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198102
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19900316
Entered Medline: 19810224

TI Lack of mutagenicity of some **phytoestrogens** in the salmonella/mammalian microsome assay.

AB 8 **phytoestrogens** were tested for mutagenicity using a variation of the Salmonella/mammalian microsome (or Ames) assay. Zearalenone is a mycotoxin produced by. . . compounds are all flavonoids which occur naturally at relatively high concentrations in many plants, particularly legumes. 4 of these flavonoids (**daidzein**, **genistein**, formononetin and biochanin-a) are **isoflavones** and the 5th, coumestrol, is a coumestan. Each compound was tested at several concentrations ranging from 1--500 micrograms per plate.. . .

CT Check Tags: Animal
*Coumarins: PD, pharmacology
Coumestrol: ME, metabolism
*Coumestrol: PD, pharmacology
*Flavones: PD, pharmacology
Isoflavones: ME, metabolism
***Isoflavones: PD, pharmacology**
Mutagenicity Tests
*Mutagens
Rats
*Resorcinols: PD, pharmacology
Salmonella typhimurium: DE, drug effects

Zearalenone: ME, metabolism
*Zearalenone: PD, pharmacology
. . .
CN 0 (Coumarins); 0 (Flavones); 0 (**Isoflavones**); 0 (Mutagens); 0 (Resorcinols)

L3 ANSWER 418 OF 540 MEDLINE on STN
ACCESSION NUMBER: 76257195 MEDLINE
DOCUMENT NUMBER: 76257195 PubMed ID: 1066275
TITLE: Occurrence of anabolic agents in plants and their importance.
AUTHOR: Lindner H R
SOURCE: ENVIRONMENTAL QUALITY AND SAFETY. SUPPLEMENT, (1976) (5) 151-8.
Journal code: 7512713. ISSN: 0340-4714.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197610
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 19900313
Entered Medline: 19761020
AB . . . Britain, Germany, Denmark, Holland, Finland, Egypt and Israel. The clover constituents chiefly incriminated for these effects are glycosides of the **isoflavone** derivatives **genistein** and its 4'-methyl ether biochanin-A, **daidzein** and its 4'-methyl ether formononetin, and pratensein; coumestrol and its 3'- and 4'-methyl ethers account for the estrogenic activity of alfalfa. The **isoflavone** content of subterranean clover may reach 3 percent of its dry weight, and the coumestrol content of lucerne may exceed 100 mug/g. Coumestrol and **genistein** compete with 17beta-estradiol for binding sites on the uterine cytoplasmic receptor and induce macromolecular synthesis in the uterus, but fail to induce ovum implantation in ovariectomized, gestagen-maintained rats. Uterotrophic activity of coumestrol and **genistein** given parenterally to sheep is approximately 10(-3) and 10(-5) times that of stilboestrol, respectively. Biological activity of ingested **phytoestrogens** is modified by ruminal micro-organisms and hepatic metabolism...
CT . . . Male
Coumestrol: ME, metabolism
Coumestrol: PD, pharmacology
*Estrogens, Non-Steroidal
Estrogens, Non-Steroidal: ME, metabolism
Estrogens, Non-Steroidal: PD, pharmacology
Infertility: VE, veterinary
Isoflavones: ME, metabolism
Isoflavones: PD, pharmacology
*Plants
Plants: AN, analysis
Rats
Sheep: GD, growth & development
Sheep: ME, metabolism
Sheep Diseases: CI, chemically. . .
CN 0 (Estrogens, Non-Steroidal); 0 (**Isoflavones**)

L3 ANSWER 419 OF 540 USPATFULL on STN
ACCESSION NUMBER: 2003:319342 USPATFULL
TITLE: Dual inhibitors of adipocyte fatty acid binding protein and keratinocyte fatty acid binding protein
INVENTOR(S): Magnin, David R., Hamilton, NJ, UNITED STATES
Sulsky, Richard B., West Trenton, NJ, UNITED STATES
Robl, Jeffrey A., Newtown, PA, UNITED STATES
Caulfield, Thomas J., Paris, FRANCE

Parker, Rex A., Titusville, NJ, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003225091	A1	20031204	
APPLICATION INFO.:	US 2002-295819	A1	20021115	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-333194P	20011116 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: STEPHEN B. DAVIS, BRISTOL-MYERS SQUIBB COMPANY, PATENT DEPARTMENT, P O BOX 4000, PRINCETON, NJ, 08543-4000
NUMBER OF CLAIMS: 21
EXEMPLARY CLAIM: 1
LINE COUNT: 4951
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM [0205] The other lipid agent also includes a **phytoestrogen** compound such as disclosed in WO 00/30665 including isolated soy bean protein, soy protein concentrate or soy flour as well as an **isoflavone** such as **genistein**, **daidzein**, glycitein or equol, or phytosterols, phytostanol or tocotrienol as disclosed in WO 2000/015201;

L3 ANSWER 538 OF 540 USPATFULL on STN
ACCESSION NUMBER: 96:40969 USPATFULL
TITLE: Dietary **phytoestrogen** in estrogen replacement therapy
INVENTOR(S): Hughes, Claude L., Mebane, NC, United States
Henley, Edna C., St. Louis, MO, United States
Clarkson, Thomas B., Clemmons, NC, United States
PATENT ASSIGNEE(S): Wake Forest University, Winston-Salem, NC, United States (U.S. corporation)
Protein Technologies International, Inc., St. Louis, MO, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5516528		19960514	
APPLICATION INFO.:	US 1995-372750		19950113	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Azpuru, Carlos
LEGAL REPRESENTATIVE: Farrell, Kevin M.
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 864
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

TI Dietary **phytoestrogen** in estrogen replacement therapy

AB Disclosed is a pharmaceutical composition for oral delivery. The composition includes about 1-2 mg mammalian estrogen and about 25-100 mg **phytoestrogen**. Compositions of the type described above are utilized, for example, in a therapeutic regimen designed to reduce the risk of. . . and osteoporosis in postmenopausal women. This method comprises the oral administration of a composition comprising a mixture of estrogen and **phytoestrogen**, the dosages of mammalian estrogen and **phytoestrogen** being about 1-2 mg, about 25-100 mg, respectively.

SUMM . . . relates to a pharmaceutical composition for oral delivery. The composition includes about 1-2 mg mammalian estrogen and about 25-100 mg **phytoestrogen**. In a preferred embodiment, the pharmaceutical composition is in pill or capsule form. The invention also encompasses transdermal or implant delivery systems calibrated to deliver estrogen and **phytoestrogen** within the previously stated ranges.

SUMM Preferably the estrogen is estradiol, and the **phytoestrogen** is a **phytoestrogen** which functions as an estrogen antagonist in breast and uterine tissue. Members of the **isoflavone** class of **phytoestrogen** (e.g., **genistein** and **daidzein**) are particularly preferred.

SUMM . . . and osteoporosis in postmenopausal women. This method comprises the oral administration of a composition comprising a mixture of estrogen and **phytoestrogen**, the dosages of mammalian estrogen and **phytoestrogen** being about 1-2 mg, about 25-100 mg, respectively.

DRWD FIG. 1 is a diagram showing the chemical structures of the soy **phytoestrogens genistein** (4',5,7-trihydroxyisoflavone) and **daidzein** (4',7-dihydroxyisoflavone) compared with estradiol of mammalian origin.

DETD . . . 12:207 (1954), and Farnsworth et al., J. Pharm. Sci. 64:717 (1954)). These compounds have been given the general name of " **phytoestrogens**" and represent several chemical classes. Similarities in the molecular structure of **phytoestrogens** facilitate binding to the estrogen receptor. An important class of **phytoestrogen** is the **isoflavone** class. The chemical structures of the soy **isoflavones genistein** (4',5,7-trihydroxyisoflavone) and **daidzein**

(4',7-dihydroxyisoflavone) are compared with estradiol of mammalian origin in FIG. 1. **Phytoestrogens** have been shown to exhibit mixed estrogen agonist-antagonist properties which are organ-specific in vivo (Setchell, In McLachlan, Ed., Estrogens in. . .

DETD Soybeans are a particularly important source or **phytoestrogen**. Several hundred varieties or cultivars of soybeans exist, and their **phytoestrogen** content can vary from 50 mg/100 g to 300 mg/100 g. In addition, given the high levels of consumption of. . . by certain Oriental cultures, there is a substantial body of relevant epidemiologic evidence. For example, there is epidemiologic evidence that **phytoestrogens** are associated with lower risk of development of breast and uterine cancer. Oriental women have lower rates of breast cancer. . . than American women (Coward et al., J. Agr. Food Chem. 41:1961 (1993)); as a result, differences in urinary excretion of **phytoestrogens** between Oriental women and American women are striking (Oriental: 2,000-3,000 nmol/24 hour of **genistein** and **daidzein**; American: 30-40 nmol/24 hour). Experimental evidence for lower breast cancer incidence associated with dietary **phytoestrogens** in soybeans has also been reported (Barnes et al., In Jacobs MM, ed., Diet and Cancer: Markers, prevention and treatment, New York: Plenum Press, 135 (1994)). Specifically, dietary soy protein preparations enriched with **phytoestrogens** inhibited mammary tumors in rats treated with 7,12-dimethyl-benz[a]anthracene compared to rats fed low-**phytoestrogen** soy protein preparations.

DETD The increasingly frequent suggestion that **phytoestrogens** may protect against development of coronary artery atherosclerosis (CAA) and CHD is based on the evidence that endogenous estrogen protects. . . hormones accounts for the progressing CHD of postmenopausal women, and that estrogen replacement decreases CHD risk among postmenopausal women. That **phytoestrogens** may be cardioprotective is supported by the low rates of CHD among Oriental people compared to Westerners, and the low. . .

DETD In addition to the epidemiological evidence, recent experimental observations have suggested that **phytoestrogens** may protect against the development of CAA. For example, studies have shown that male casein-fed rats had significantly higher total plasma cholesterol (TPC) and low density lipoprotein cholesterol (LDL-C) concentrations than soy protein-fed rats. When soybean **phytoestrogens** were added to casein, the animals had LDL-C concentrations similar to the soy protein-fed group.

DETD In premenopausal female monkeys fed soy protein enriched in **phytoestrogens**, TPC concentrations were significantly lower and HDL-C concentrations were higher among **phytoestrogen**-enriched soy protein-fed animals. Furthermore, among surgically postmenopausal female monkeys, HDL-C concentrations were higher (50 versus 67 mg/dl), while TPC concentrations were similar between **phytoestrogen** -enriched and low-**phytoestrogen** soy-fed groups.

DETD Finally, among the **phytoestrogens**, only **genistein** (the principal **phytoestrogen** of soybeans) has been the subject of numerous cellular/molecular biologic studies suggesting its potential beneficial effects of several key aspects. . .

DETD TABLE 1

Genistein Effects	Reference
Inhibits interleukin-2 and leukotriene B4 production by mononuclear cells	Atluru et al., Clin. Immunol. Immunopathol. 59:379 (1991)
Inhibits platelet-derived growth	Fujio et. . .

DETD . . . addition to the evidence discussed above in connection with CHD

and CAA, there is also epidemiologic and experimental evidence that **phytoestrogen** can prevent postmenopausal bone loss and osteoporosis. With respect to the epidemiologic evidence, differences in hip fracture incidence between U.S. women (low **phytoestrogen** consumers) and Asian women (high **phytoestrogen** consumers) can not be explained by the usual relationships with calcium consumption. More specifically, the per capita calcium consumption of. . .

DETD With regard to the experimental data, a recent report discloses that a low dose, but not a high dose of **genistein** was equivalent to conjugated equine estrogen (CEE) in maintaining bone mass in ovariectomized rats. Several reports have provided further evidence for a bone-protective effect of **genistein**. These reports focused on a structurally similar compound, ipriflavone, which is a synthetic 7-isopropoxyisoflavone (Gambacciani et al, J. Endocrinol. Invest.. . .

DETD The epidemiologic and experimental results discussed above are consistent with a hypothesis that dietary **phytoestrogen** represents a critical element in the diet of Oriental women which is, at the very least, non-antagonistic of the positive. . . and uterine tissue. If true, this hypothesis suggests that a dietary regimen designed to produce serum levels of estrogen and **phytoestrogen** mimicking those of premenopausal Oriental women represents a better alternative to current estrogen replacement therapy. More specifically, the known risks of increased incidence of breast cancer associated with hormone replacement therapy would be reduced by the antagonist effects of **phytoestrogen** in breast tissue. Similarly, in uterine tissue, the antagonistic effects of **phytoestrogen** would result in a reduction in the risk of endometrial cancer relative to conventional estrogen replacement therapy. Furthermore, this estrogen. . .

DETD . . . specifically, Wilcox et al. (Br. Med. J. 301:905 (1990)) reported increases in vaginal cell proliferation among postmenopausal women consuming soybean **phytoestrogens** for 6 weeks. In addition, Markiewicz et al. (J. Steroid Biochem. 45:399 (1993)) demonstrated experimentally that the soy **isoflavone genistein** exhibited an estrogen effect on endometrial cancer cells in an in vitro bioassay. These reports are in conflict with the. . .

DETD As shown in Example 1, the soy **isoflavone genistein** does not act as an estrogen on the vaginal epithelium. The statistically significant data reported in Example 1 supports the epidemiological and experimental results which indicate that a diet which mimics the diet of Oriental women with respect to **isoflavone** consumption will reduce the known risks of increased incidence of breast cancer associated with hormone replacement therapy by virtue of the antagonist effects of **phytoestrogen** in breast tissue. The data also supports the epidemiological and experimental results which indicate that a diet which mimics the diet of Oriental women with respect to **isoflavone** consumption will reduce the risk of endometrial cancer by virtue of the antagonistic effects of **isoflavone** resulting in a reduction in the risk of endometrial cancer relative to conventional estrogen replacement therapy. Furthermore, this estrogen antagonistic. . .

DETD . . . the invention relates to a pharmaceutical composition for oral delivery comprising about 1-2 mg mammalian estrogen and about 25-100 mg **phytoestrogen**. This composition would be administered orally to postmenopausal women. Preferably, the composition would be formulated in pill or capsular form. . . using conventional manufacturing techniques. The preferred mammalian estrogen is estra-1,3,5(10)-triene-3,17.beta.-diol, commonly known as estradiol (e.g., Estrace.RTM., Mead Johnson). The preferred **phytoestrogen** is selected from the isoflavanoid group. In particular, the isoflavanoids **genistein** and **daidzein** have been discussed previously. Transdermal and implant delivery systems calibrated to deliver about 1-2 mg estrogen and about 25-50 mg **phytoestrogen**/day are also encompassed by the

present invention.

DETD The administration of about 1-2 mg mammalian estrogen and about 25-100 mg **phytoestrogen** to postmenopausal women on a daily basis will serve to decrease the risk of osteoporosis and coronary heart disease, without. . .

DETD . . . two dietary treatments: CEE at a dose equivalent on a caloric basis to 0.625 mg/woman/day; or SBE at 1.27 mg **genistein** per g protein. In the second study (study two), 116 surgically postmenopausal adult female cynomolgus macaques were randomized into 5. . .

DETD . . . of estradiol and estrone. Tamoxifen, an estrogen antagonist in breast tissue, has estrogenic effects on the vaginal epithelium. SBE containing **genistein**, does not act as an estrogen on the vaginal epithelium. MPA neutralizes the effects of CEE and has no estrogenic. . .

DETD . . . the maturation index or percent superficial cells in vaginal smears. The concentrations of serum estradiol and urinary soy estrogens (urinary **daidzein**, **genistein**, and equol) were also measured at baseline and at the end of the diet-intervention period. All laboratory personnel were blinded. . .

DETD . . . the local health food store, were provided as a daily snack. The soybeans, TVP, and soy splits were analyzed for **daidzein** and **genistein** by HPLC-mass spectrometry, as described previously (Setchell et al., Gastroenterology 93:225 (1987)). The daily intake of soy consisted of 38 gms of dry TVP (2.1 mg/g **daidzein** , 0.6 mg/g **genistein**) or 114 gms of dry whole soybeans (0.7 mg/g **daidzein**, 0.2 mg/g **genistein**). In addition, women ate 25 gms of soy splits daily (1.8 mg/g **daidzein**, 0.7 mg/g **genistein**). Thus, daily intake of **isoflavones** was 165 mg/day. This is approximately equivalent on a molar basis to 0.3 mg/day of conjugated steroidal estrogen assuming that the estrogenic activity of the **phytoestrogens** is about 0.1% that of conjugated estrogen.

DETD Urinary **phytoestrogens** (**daidzein**, **genistein** , and equol) were measured simultaneously in 5 ml. aliquots of each urine specimen. **Isoflavones** were extracted from urine by solid-phase extraction after addition of an internal standard 5.alpha.-androstane-3.alpha., 17.alpha.-diol (5 .mu.g). Conjugates were hydrolyzed. . .

DETD Urinary concentrations of soy **isoflavones** were measured to demonstrate compliance with the diet and to provide a crude measure of **phytoestrogen** dose for each participant. Earlier work (Setchell et al., Am. J. Clin. Nutr. 40:569 (1984)) had shown that urinary equol. . . by the third or fourth day after cessation of the diet. To minimize the effect of day-to-day variations in urinary **isoflavone** levels, first morning urine samples from before the diet (6 ml. aliquots from each of the seven days before randomization). . . diet period (2 ml. aliquots from each day of the last three weeks of the diet) were pooled prior to **phytoestrogen** measurement in the pooled sample. Concentrations were expressed relative to the creatinine concentration in the pooled sample. A pilot study of 20 paired specimens had been conducted to measure **phytoestrogen** concentrations in 24-hour and first-morning urine specimens from the same 24-hour period to verify that first-morning urine specimens (corrected for. . .

DETD . . . urinary soy estrogens: equol concentrations alone, an unweighted sum, or a weighted sum of concentrations with weights of 4 for **daidzein**, 8 for **genistein**, and 100 for equol, based on laboratory data on their relative estrogenicity (Shutt, D.A., and Cox, R.I., J. Endocrinol. 52:299. . .

DETD . . . The two who were assigned to the soy diet group had low baseline levels and showed large increases in urinary **isoflavone** concentrations during the diet period.

DETD This Example relates to experiments designed to evaluate the beneficial affects of a combined estrogen/**phytoestrogen** dietary regimen

on rats. More specifically, serum lipid profiles, as well as effects on the uterus and bone will be. . .

DETD TABLE 2

Group	Diet
1. Low **Phytoestrogen** + Sham OVX	Low-**isoflavone** soybean-based diet (equivalent on a caloric basis to 12.05 mg **Genistein**/woman/day), intact female
2. Low **Phytoestrogen** + OVX	Low-**isoflavone** soybean-based diet equivalent to the above diet, ovariectomized female
3. Low **Phytoestrogen** + OVX + Low Premarin	Low-**isoflavone** soybean-based diet + Premarin equivalent on a caloric basis to the above diet with the addition of 0.3125 mg Premarin/woman/day
4. Low **Phytoestrogen** + OVX + High Premarin	Low-**isoflavone** soybean-based diet + Premarin equivalent on a caloric basis to the above diet with the addition of 0.625 mg Premarin/woman/day
5. High **Phytoestrogen** + Sham OVX	High-**isoflavone** soybean-based diet (equivalent on a caloric basis to 117.06 mg **Genistein**/woman/day), intact female
6. High **Phytoestrogen** + OVX	High-**isoflavone** soybean-based diet, ovariectomized female
7. High **Phytoestrogen** + OVX + Low Premarin	High-**isoflavone** soybean-based diet + Premarin equivalent on a caloric basis to the above diet with the addition of 0.3125 mg Premarin/woman/day
8. High **Phytoestrogen** + OVX + High Premarin	High-**isoflavone** soybean-based diet + 0.625 mg Premarin/woman/day
9. Casein + Sham OVX	Casein-based diet, intact female, no Premarin
10.. . .	

DETD . . . to the dose which would be arrived at by scaling on the basis of body surface area. The human equivalent **isoflavone** intake has been estimated by the same equation. Purified diets will be formulated by the Comparative Medicine Clinical Research Center Diet Laboratory. Sufficient **isoflavone**-rich and **isoflavone** -poor soy protein is on hand to formulate all diets needed for this study.

DETD 1. Premarin with **phytoestrogen** will result in lower levels of endometrial hyperplasia in rats relative to Premarin with casein.

DETD 2. Premarin with **phytoestrogen** will retain the bone loss prevention effects of Premarin alone.

DETD 3. Casein alone will result in loss of bone mass relative to Premarin

and **phytoestrogen** treated groups.

DETD 4. **Phytoestrogen** diet alone will not cause endometrial hyperplasia relative to Premarin-treated and casein-fed controls.

DETD . . . in this example are intended to examine the separate and combined effects of a traditional estrogen (estradiol) and a soybean **phytoestrogen** on coronary artery function and reproductive organ pathology in the cynomolgus monkey. Fifty-six adult female cynomolgus monkeys will be selected. . .

DETD . . . LH and FSH) will be determined once prior to treatment and then 2 months and 5 months after treatment. Plasma **genistein** concentrations will be measured once, 5 months after treatment. Vaginal Cytology will be done 2 and 5 months after treatment,. . .

CLM What is claimed is:

1. A pharmaceutical composition for oral delivery comprising a combination of mammalian estrogen and soy-derived **phytoestrogen** in an amount sufficient to reduce the risk of coronary heart disease and osteoporosis in women.

. . . of coronary heart disease and osteoporosis in women is about 1-2 mg of mammalian estrogen combined with about 25-100 mg **phytoestrogen**.

5. A pharmaceutical composition of claim 1 wherein the **phytoestrogen** is an **isoflavone**.

6. A pharmaceutical composition of claim 5 wherein the **isoflavone** is **genistein**.

7. A pharmaceutical composition of claim 5 wherein the **isoflavone** is **daidzein**.

8. A method for reducing the risk of coronary heart disease and osteoporosis in women, the method comprising administering orally a combination of mammalian estrogen and soy-derived **phytoestrogen** in a therapeutically effective amount.

. . . claim 8 wherein the therapeutically effective amount is a daily dosage of mammalian estrogen of about 1-2 mg, and soy-derived **phytoestrogen** of about 25-100 mg.

11. A method of claim 8 wherein the soy-derived **phytoestrogen** is an **isoflavone**.

12. A method of claim 11 wherein the **isoflavone** is **genistein**.

13. A method of claim 11 wherein the **isoflavone** is **daidzein**.

14. An estrogen replacement therapy regimen, comprising the oral coadministration of a combination of mammalian estrogen and soy-derived **phytoestrogen** in a therapeutically effective amount.

. . . claim 14 wherein the therapeutically effective amount is a daily dosage of mammalian estrogen of about 1-2 mg, and soy-derived **phytoestrogen** of about 25-100 mg.

17. A method of claim 14 wherein the soy-derived **phytoestrogen** is an **isoflavone**.

18. A method of claim 17 wherein the **isoflavone** is **genistein**.

19. A method of claim 17 wherein the **isoflavone** is

daidzein.

L3 ANSWER 539 OF 540 USPATFULL on STN
ACCESSION NUMBER: 96:29540 USPATFULL
TITLE: **Genistein** for use in inhibiting osteroclasts
INVENTOR(S): Barnes, Stephen, Birmingham, AL, United States
Blair, Harry C., Mountain Brook, AL, United States
PATENT ASSIGNEE(S): The UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5506211		19960409	
APPLICATION INFO.:	US 1994-241040		19940509	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Dees, Jose G.
ASSISTANT EXAMINER: Frazier, Barbara S.
LEGAL REPRESENTATIVE: Arnold, White & Durkee
NUMBER OF CLAIMS: 27
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 23 Drawing Figure(s); 23 Drawing Page(s)
LINE COUNT: 1696
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

TI **Genistein** for use in inhibiting osteroclasts

AB . . . use in inhibiting osteoclast activity and, particularly, the osteoclast acid secretion that leads to bone-degradation. It is shown that the **isoflavone genistein** inhibits the acid secretion of osteoclasts and reduces bone resorption. The present invention thus provides advantageous methods for use in. . .

SUMM The present invention relates generally to the fields of bone cells and also to **isoflavones**. Particularly, the invention concerns the discovery that the **isoflavone genistein** inhibits the acid secretion of bone-degrading cells termed osteoclasts. The invention thus provides methods and compositions for use in inhibiting. . .

SUMM . . . the prior art by providing new methods for use in reducing acid secretion by osteoclasts. The inventors discovered that the **isoflavone genistein**, found in soy products, effectively inhibits acid secretion and osteoclastic bone resorption. The invention thus provides methods for use in. . .

SUMM . . . present invention, one would generally contact one or more osteoclasts with a composition that comprises a biologically effective amount of **genistein**. "**Genistein**" as used herein refers to the **isoflavone** compound as described in the Merck Index (7th Edition, 1960, p 474), or a derivative or analogue thereof that functions as a tyrosine kinase inhibitor. The ability of a **genistein** analogue to inhibit tyrosine kinase, and particularly, to inhibit pp60.sup.src, may be readily determined by methods known to those of. . .

SUMM The present invention thus encompasses the use of any **genistein** derivative that has a significant (i.e., consistently above background) inhibitory effect on tyrosine kinases. Also encompassed are **genistein**-derived compounds that may be formed upon ingestion. For example, **genistein** glucuronides, as for other glucuronides, are hydrolyzed by .beta.-glucuronidases in the large bowel, and the unconjugated forms reabsorbed. In this environment the **genistein** compounds may undergo other chemical modifications, such as reduction to isoflavans or B-ring-opened forms, all such compounds are intended to. . .

SUMM The term "a biologically effective amount", as used herein, refers to an amount of purified **genistein**, or an amount of a **genistein**-containing composition, that is effective to inhibit osteoclast acid secretion. As disclosed herein in Examples I, II and IV,

and in FIGS. 1-4, **genistein** is effective at inhibiting osteoclast acid secretion at levels of less than 1 .mu.M (0.27 .mu.g/ml), with half maximal inhibition. . . with at about .about.2 .mu.M, with a mean of about 3 .mu.M (0.75 to 0.8 .mu.g/ml). Therefore, amounts of purified **genistein** or **genistein**-containing compositions that result in local concentrations of **genistein** in approximately these ranges are "effective amounts".

SUMM . . . bone resorption assay uses material that includes all bone cells, it is an ex vivo assay. Thus, the showing that **genistein** inhibits bone resorption in these assays is evidence of the clinical utility of **genistein** for treating osteoporosis. Various scientific publications, such as Carano et al. (1990); Blair & Schlesinger (1992); Schlesinger & Blair (1992);. . .

SUMM Although the advantageous results disclosed herein clearly teach that **genistein** has utility in the inhibition of bone resorption, and may thus be used to treat osteoporosis in humans, several other. . . particularly, the treatment of osteoporotic fractures in poultry, which poses a major problem in the egg production industry. Also, as **genistein** inhibits osteoclastic acid secretion, it may thus be used in vitro, for example, in connection with tissue culture methods and. . .

SUMM . . . certain embodiments, osteoclasts located within an animal, such as a dog, horse, chicken or human subject, may be contacted with **genistein**, thereby inhibiting their activity and acid secretion in vivo. To achieve this, a purified **genistein** compound, or equally, a **genistein**-containing composition or food, would be administered to the animal. Any **genistein**-containing composition may be used to achieve in vivo effects, so long as it is in a pharmaceutically acceptable form. The. . .

SUMM Preparing **genistein** in a pharmaceutically acceptable form, or obtaining a **genistein**-containing composition that is pharmaceutically acceptable, does not pose a problem. In fact, it is an advantage of this invention that **genistein** is present within a variety of foodstuffs, particularly soy products, and that such foods, or concentrated forms thereof, may simply be ingested to provide an animal with an effective amount of **genistein**. Indeed, populations of Southeast Asians eat 70 mg of **genistein** per day without obvious ill effects (Soybean Utilization, Eds. Snyder & Kwon, p. 220). This is also a cost-effective method as isolated soy protein, containing about 450 mg of **genistein** per 450 gm (pound), can be bought cheaply. At current prices, a gram of **genistein** obtained within isolated soy protein in the U.S. costs approximately $3.75.

SUMM However, it is equally possible to provide **genistein** to an animal or human in a purified form, particularly a tablet. Therefore, **genistein**, or an inhibitory **genistein** derivative or analogue, may be obtained in a more purified form and administered to an animal, either orally, or via virtually any other route, such as intravenous injection. The formulation of **genistein** into a tablet is particularly preferred in certain embodiments, as this provides a simple means for ingesting **genistein** that is acceptable to the patient. Methods for purifying **genistein** are disclosed herein in Examples VI and VII. Also, synthetic **genistein** may be purchased from many commercial sources, such as, e.g., Gibco, L. C. Services and Upstate Biotechnology Incorporated (UBI).

SUMM Furthermore, owing to a present inventor's discovery that **isoflavones** in soy are present as malonylglucosides, it is proposed that such charged species may be advantageously purified using an anion. . . acetone solvents. The purification of malonylglucosides would result in the added benefit of directly providing one of the more preferred **genistein** conjugates.

SUMM Whether **genistein** is administered in a purified or semi-purified form, particularly a tablet, or is administered as part of

an unmodified foodstuff, it is contemplated that preferred forms of **genistein** for use in treatment protocols will be those in which the **isoflavone** component is conjugated to an organic acid, such as the 6"-O-acetylglucosides and 6"-O-malonylglucosides. The reason for this is that the inventors have discovered that conjugated forms of **genistein** are absorbed in the large bowel and thus have advantageous biodistribution properties.

SUMM Certain methods for administering **genistein** to an animal or patient include giving the patient an ingestible soy food product. In general, any soy product may be used and, as prior heating does not affect the activity of **genistein** once absorbed, even those soy compositions that have been heated or otherwise processed, may be employed. These include products such. . .

SUMM . . . its derived products such as FIRST ALTERNATIVE.TM., a low-fat soy mil, and a low-fat tofu termed MORI-NU. Further examples are **genistein** conjugates that have been purified or semi-purified, or even synthesized, and formulated into a concentrated material, such as a tablet. Tablets that contain about the recommended daily **genistein** dose of between about 2-50 mg to 20-50 mg **genistein**, as described below, would be preferred, or ones that contain half or a third of such a dose, which would. . .

SUMM . . . disease. The treatment methods of the invention generally comprise administering to such an animal or human subject a pharmaceutically acceptable **genistein** composition in an amount that is effective to reduce acid secretion by osteoclasts located within the animal. Suitable **genistein** compositions include all soy food products, and more preferably, those containing **genistein** in a conjugated form, such as unprocessed soy food products, those soy compositions isolated without significant heating, and also tablets or other concentrated forms of purified or synthetic **genistein** and **genistein** conjugates.

SUMM Amounts of **genistein**-containing compositions that are effective to reduce acid secretion by osteoclasts in vivo are also termed "pharmacologically effective amounts". The appropriate pharmacological doses of **genistein** (whether purified or present within a soy food) for use in treating osteoporosis, or other bone resorption disorders, may be. . .

SUMM . . . the data presented in Examples I through V, it is contemplated that to produce a sufficiently high (i.e., effective inhibitory) **genistein** concentration at the osteoclast, a daily intake of about 2-50 mg of **genistein** would be required for a human patient. More preferably, such doses would be between about 5-50 mg, about 10-50 mg,. . .

SUMM . . . osteoclasts would likely be compensated for by the production of parathyroid hormone (PTH). As disclosed herein, the therapeutic margin for **genistein** is particularly good, with toxic effects not being observed until about a 30-fold excess over the half maximal inhibitory effects.. . . practiced in the art. One would particularly take note of the patient's body weight, age, and health, as well as **genistein** uptake, **genistein** secretion, serum **genistein** levels and other factors well known to those in the medical arts, such as, e.g., liver function. One would also. . .

SUMM In terms of human treatment, certain suitable methods include administering from 2-50 mg to 20-50 mg of **genistein** in the form of a food product. This may be achieved by ingesting between about 2 g to 50 g,. . . to about 50 g, of soy isolated soy protein, per day per person. This daily intake of 20-50 mg of **genistein** could also be obtained from 2-40 or 20-40 g of unheated soy flours, soy protein concentrate, textured soy, or from. . . patient is not recommended. An equally suitable treatment method is the administration of from 2-50 mg to 20-50 mg of **genistein** in the form of one or more tablets.

DRWD FIG. 1. **Genistein** inhibits osteoclastic bone degradation. Bone degradation assays were conducted as described in Example I.

Genistein significantly inhibits bone degradation. Control note: the **genistein** congener dadzein, which is not an inhibitor of tyrosine kinases, has no effect on bone resorption at 10 .mu.M, and. . .

DRWD FIG. 2. **Genistein** inhibits osteoclast cell membrane acid transport. This figure shows that at doses comparable to those inhibiting bone resorption by whole cells, i.e., those shown in FIG. 1, osteoclast cell membrane acid transport is inhibited. Dadzein, a **genistein** congener that does not inhibit tyrosine kinases, was used as a control. It was found that dadzein has no effect. . .

DRWD FIG. 3. Osteoclasts survive after removal of **genistein**. The same methods were employed as those used to generate the data in FIG. 1. This figure shows that after exposure to moderate levels of **genistein** for 48 hours, functional osteoclasts may be recovered after **genistein** washout. It should be noted that at 30 .mu.M **genistein**, cells die in 2-3 days.

DRWD FIG. 4. **Genistein** inhibition of osteoclast acidification is proportional to the inhibitor concentration. This figure shows that osteoclast acidification is proportional to the inverse of the inhibitor concentration (thus more **genistein** results in less acid), suggesting that one important chemical interaction is involved.

DRWD FIGS. 5A and 5B. Reversed-phase HPLC analysis of **isoflavones** in soy protein isolate extracted with 80% aqueous methanol at room temperature (A), or at 80.degree. C. for 4 h (B). Peak identification: 1=dadzin, 2=glycitin, 3=genistin, 4=**daidzein** 6-OMalGlc, 5=glycitein 6-OMalGlc, 6=**daidzein** 6-OAcGlc, 7= **genistein** 6-OMalGlc, 8=**genistein** 6-OAcGlc, 9= **genistein**, 10=fluorescein internal standard. Analyses were performed on a 25 cm.times.4.6 mm C.sub.8 Aquapore column using a linear elution gradient of 0-50% acetonitrile in 0.1% trifluoroacetic acid over 30 min at a flow rate of 1.5 ml /min. **Isoflavones** were detected by their absorbance at 262 nm.

DRWD FIGS. 6A and 6B. Reversed-phase HPLC analysis of **isoflavones** extracted from toasted soy flour with 80% aqueous methanol at room temperature for 2 hours (A) and at 80.degree. C.. . .

DRWD FIGS. 7A, 7B and 7C. Selected positive ion chromatograms of **genistein** glycosidic conjugates in isolated soy protein (A), toasted soy flour (B), and defatted soy flour (C) following HPLC-APCI-HN analysis on. . . 0-50% acetonitrile gradient over 10 min in 2 mM ammonium acetate at a flow rate of 1 ml/min. Peak identification: 1= **genistein** 6-OMalGlc, 2=genistin, 3=**genistein** 6-OAcGlc. The individual analyses were performed over a six month period, accounting for the small differences in retention times.

DRWD FIGS. 8A, 8B and 8C. Selected positive ion chromatograms of **genistein** glycosidic conjugates in soybean hypocotyl (A), tofu (B) and soy molasses (C) following HPLC-APCI-HN analysis on a 10 cm.times.4.6 mm. . .

DRWD FIGS. 9A, 9B and 9C. Positive ion mass spectra of **daidzein** (A), **genistein** (B), and glycitein (C) 6-OMalGlc conjugates in isolated soy protein separated by reversed-phase HPLC.

DRWD FIGS. 11A, 11B and 11C. Positive ion mass spectra of 6-OAcGlc conjugates of **daidzein** (A), **genistein** (B), and glycitein (C) in isolated soy protein separated by reversed-phase HPLC.

DETD **Genistein**, 5,7,4'-trihydroxyisoflavone, is one of the **isoflavones** found in soy. Certain reports have speculated on the presence of **genistein** and other **isoflavones** in soy oil or sauce, such as the abstract from the Japanese patent application JP 5170756. However, the present inventors have demonstrated that soy oil or sauce does not contain measurable, let alone isolatable, amounts of **isoflavones**, as described in Example VI, Table III, and in Coward et al. (1993).

DETD . . . report (JP Application 5170756) also suggests that 500 g of defatted soybeans can be extracted to yield 9.5 g of **isoflavone** aglucones at a purity of 90%. However, this figure is in marked contrast

to the generally held belief in the art as to the quantity of **isoflavones** in soy products. For example, the present inventors have shown that the total **isoflavone** content of soy flour is about 2.7 mg/g, i.e., a maximum of about 1.35 g in 500 g, see Example. . . VI and Coward et al. (1993). This is supported by other papers (e.g., Eldridge, 1982; Murphy, 1982) that give the **isoflavone** concentrations in soy as being between about 1 mg/g and about 3 mg/g.

DETD **Genistein** is known to be a tyrosine kinase inhibitor (Akiyama et al., 1987) and has been proposed for use in treating. . .

DETD . . . are bone-anabolic, i.e., have effects on osteoblasts (Brandi, 1992). The abstract from the patent application WO 93/23069 also suggests that **genistein** may be used to treat adverse symptoms associated with menopause in women. This document, however, concentrates on the estrogen-like actions of **genistein**, referring to the treatment of breast cancer and pre-menstrual tension, and does not appear to mention osteoporosis.

DETD Although **genistein** is known to inhibit tyrosine kinases, it is chemically unrelated to Herbimycin A. It is also important to note that there have been articles published in the scientific literature that argue against the use of **genistein** in bone resorption. For example, in papers that concern the actions of **genistein** on bone tissues (Davidai et al., 1992; Quarles et al., 1993; Schvartz et al., 1992), **genistein** is shown to inhibit stimulators of osteoblast function. As osteoblasts, as opposed to osteoclasts, are bone-forming cells, this data indicates that **genistein** has an adverse effect on bone and suggests that **genistein** may even cause osteoporosis.

DETD Despite suggestions to the contrary, the inventors contemplated that **genistein** may inhibit osteoclast activity and may prove useful in treating bone disorders. In testing the effects of **genistein** on osteoclasts, it was found that **genistein** was indeed able to inhibit osteoclast acid secretion, to alter the ability of osteoclasts to bind to bone, and to inhibit bone resorption. Thus, the present invention includes the use of **genistein** to reduce or prevent bone loss, such as in ageing (osteoporosis) and cancer progression (metastatic bone disease).

DETD The data presented herein showing that **genistein** targets a central metabolic activity of the bone resorbing osteoclast by inhibiting acid secretion also suggests that **genistein** functions via a different mechanism to herbimycin. Herbimycin appears to primarily effect chloride conductivity in the ruffled border (specifically, its. . . by by-passing chloride conductivity using the potassium ionophore valinomycin (2 .mu.M) in the presence of potassium (120 mM). In contrast, **genistein** inhibits acid transport by a different mechanism that is not reversed by falinomycin under these conditions.

DETD An **isoflavone** termed ipriflavone (7-isopropoxylisoflavone) has been used in clinical trials for the treatment of postmenopausal and senile osteoporosis. However, it is important to note that the active form in vivo is not known and that one of its metabolites, **daidzein** (4',7-dihydroxyisoflavone) has negligible activity in the osteoclast assay systems disclosed herein. Thus the use of **genistein** is distinct from the use of ipriflavone.

DETD The present inventors, owing to a discovery relating to the various conjugated forms of **genistein**, also propose that certain **genistein**-containing compositions would be preferred for use in all therapeutic embodiments, including the treatment of disorders associated with bone resorption. As described below, for biodelivery purposes, the inventors prefer to use a soy composition that comprises **genistein** conjugated to an organic acid. It is an added advantage that the most preferred forms may be easily taken orally,. . .

DETD Except for heavily fermented soy-based foods such as miso, **isoflavones** in most of the common soy foods (soy milk, tofu, soy

flour, soy protein concentrate and soy protein isolate) are present as glycosidic conjugates. However, the present inventors have found that in the unprocessed soybean hypocotyl and cotyledon, the **isoflavones** are found as .beta.-glucosides (.beta.Glc), 6-O"-malonylglucosides (6OMalGlc) and 6-O"-acetylglucosides (6OAcGlc). In the past, recovery of **isoflavones** from these food matrices has been carried out by Soxhlet extraction or by simple mixing with heated aqueous alcoholic solvents. In such extracts, the **isoflavones** were shown to be .beta.-glucoside conjugates by HPLC and subsequent-mass spectrometry.

DETD In particular, the inventors have found that **genistein**, when conjugated to an organic acid, is not readily hydrolysed in the stomach and, hence, is not available for significant absorption in the small intestine (ileum). This means that this form of **genistein** is mainly absorbed through the large bowel. Evidence for this comes from the fact that following consumption of a product high in the 6"-O-malonylglucoside conjugates of **isoflavones**, the **isoflavones** are not detected in the urine or serum from 4-8 hours later, thus indicating that the principal site of intestinal. . . bowel. On the other hand, consumption of a full-flat soy milk (containing .beta.-glucoside conjugates only) led to the detection of **isoflavones** in the serum within 2 hours and in the 0-4 hr urine collection, suggesting that absorption occurs in this case. . .

DETD In light of these data, the inventors propose that using soy compositions that have higher quantities of conjugated **genistein**, i.e., non heat-treated soy products or isolated soy protein, allows **genistein** bioavailability to be controlled and **genistein** to be targeted to the large bowel. This is envisioned to be of importance in treating bowel cancer, wherein the use of a soy composition high in conjugated **genistein** would be a significant advantage.

DETD **GENISTEIN** INHIBITS BONE RESORPTION

DETD This example shows that **genistein** inhibits bone resorption. The assay systems used in Examples I and II include both cellular assays systems and also bone. . .

DETD The effect of **genistein** on bone resorption was determined using the bone resorption assay described above. It was found that the addition of **genistein** significantly inhibited bone resorption (FIG. 1). It should also be noted that the **genistein** congener dadzein, which is not an inhibitor of tyrosine kinases, has no effect on bone resorption at 10 .mu.M and. . .

DETD **GENISTEIN** INHIBITS OSTEOCLAST ACTIVITY

DETD This example shows that the **genistein**-mediated inhibition of bone resorption, described in Example I, is achieved via the inhibition of osteoclast activity. Osteoclastic resorption, such as. . . depends on acidic degradation, which in turn depends on calmodulin and tyrosine kinase dependent intracellular regulation. Therefore, the effects of **genistein** on osteoclastic cell membrane acid transport were next analyzed.

DETD . . . that at doses comparable to those inhibiting bone resorption by whole cells, osteoclast cell membrane acid transport is inhibited by **genistein** (FIG. 2). It was also found that the **genistein** congener dadzein, which is not an inhibitor of tyrosine kinases, has no effect on osteoclast acid transport at 10 .mu.M. . .

DETD Using the same assays used to generate the data in FIG. 2, it was also found that the effects of **genistein** on osteoclast acidification are proportional to the inverse of the inhibitor concentration (FIG. 4). This suggests that one important chemical. . .

DETD OSTEOCLAST RECOVERY AFTER **GENISTEIN** REMOVAL

DETD . . . protocols, an assessment of the potential toxicity is important. This example describes results of an assay indicating that toxicity of **genistein** is unlikely to be a problem in treating osteoporosis.

DETD . . . to generate the data in FIG. 1, it was found that after a 48 hour incubation with moderate levels of **genistein**, osteoclasts

could later be recovered after the **genistein** was washed out (FIG. 3). At 30 .mu.M **genistein**, it was found that cells die in 2-3 days. Since advantageous effects are seen at 1 .mu.M, with half maximal. . .

DETD Furthermore, evidence that toxicity is highly unlikely to be a significant problem comes from the fact that **genistein** is a naturally occurring compound in some foods and that populations of Southeast Asians eat 70 mg/day without obvious ill. . .

DETD COMPARATIVE EFFECTS OF **GENISTEIN** AND HERBIMYCIN

DETD It was found that, in addition to **genistein**, Herbimycin A also inhibited acid transport. Half maximal inhibition was found at .about.10 .mu.M for **genistein** and at about .about.2 .mu.M for Herbimycin A (but note that herbimycin is about ten times more toxic).

DETD . . . the presence of 5 .mu.M herbimycin, suggesting that addition of this compound inactivates the chloride channel. In contrast, 20 .mu.M **genistein** inactivated vesicles were valinomycin insensitive. Tyrphostin A47 (PDGF/EGF selective) was also inhibitory, with half maximal inhibition at 12 .mu.M. Tyrphostin A25 (EGF/Insulin selective) and the control compounds **daidzein** (a **genistein** congener) and Tyrphostin A1 were inactive at 25 .mu.M.

DETD . . . substrate and 10.sup.4 isolated arian osteoclasts, as described in Example I, showed that all of the tryphostins, including A47 and **daidzein**, were negative to >20 .mu.M. Herbimycin and **genistein** were found to be inhibitory with half maximal effects at 0.3 to 0.5 .mu.M and about 2 to about 3. . .

DETD Despite the effective dose of herbimycin on osteoclast acid secretion being about 10 fold lower than that for **genistein**, the toxicity threshold for herbimycin is also about 10 fold lower, so these compounds appear to have similar therapeutic safety. . .

DETD THE USE OF **GENISTEIN** TO STIMULATE BONE RESORPTION IN VIVO

DETD From the data presented above, the inventors have determined that **genistein** inhibits osteoclast acid transport with half maximal inhibition at .about.2-3 .mu.M (a mean of about 3 .mu.M); and that **genistein** inhibits bone resorption with half maximal effect at 10 .mu.M, essentially being complete within 3 days at 20 .mu.M. This data can be used with other known parameters to define the therapeutically effective ranges of **genistein** and soy products for use in treatment.

DETD Adlercreutz et al. (1993) reported plasma total **genistein** concentrations as high as 0.1 .mu.M in some vegetarian women; with **genistein** plasma levels of up to 1-4 .mu.M in individuals on a high soy containing diet also being reported (Adlercreutz; Setchell; International Symposium on **Phytoestrogens**, 1993).

DETD Reasonable estimates for the plasma level of **genistein** can be also be calculated from a consideration of dietary intake and rates of metabolism and excretion. A person consuming. . . soybeans (the average amount consumed by Taiwanese; Soyatech Survey 1991) has an intake of approximately 50 mg (185 .mu.mol) of **genistein**, mostly in the conjugated form.

DETD If a dose of 70 mg (259 .mu.moles) of **genistein** (in whatever form, unconjugated, i.e., isolated or synthesized, or conjugated, i.e., in a food matrix) is ingested per day by. . .

DETD However, **genistein** is relatively hydrophobic and will be taken up by cells--which must be accounted for. Previous studies on the tissue distribution of **daidzein** (Yueh & Chu 1977) have shown that in most tissues it is in similar concentrations to those in blood. Therefore, assuming that **genistein** equilibrates with total body water (56 liters), the equilibrium blood and tissue concentration would be a maximum of 4-5 .mu.moles/liter,. . . would be reduced by metabolism and excretion and be increased by repetitive daily dosing. Since there is no evidence that **genistein** has an extended half-life in the body, the effects of carry over from day-to-day are expected to be minimal and. . .

DETD It is also known that **isoflavone** concentrations in soy are

between about 1 mg/g and about 3 mg/g (Eldridge, 1982; Murphy, 1982). Therefore, taking **genistein** concentration in soy protein foodstuffs as being a minimum of 1 mg per gram is a conservative estimate. Accordingly, the maximum daily delivery dose of **genistein** in the form of food is 80 mg in women and 100 mg in men (based on a protein intake of 80 g and 100 g per day for women and men). If **genistein** is administered separate from the soy food matrix or as a food product that is enhanced with respect to **genistein**, higher does levels can be achieved. It should also be noted here that the synthetic **isoflavone**, ipriflavone, is currently administered at a dose of 600 mg per person per day.

DETD The low aqueous solubility of **genistein** (<50 .mu.g per ml) should also be considered. This might result in a maximum total absorption independent of dose. However, by administering **genistein** mixed with the bile salt, sodium taurocholate (NaTC), its aqueous solubility can be substantially enhanced. The present inventors have used this delivery method for studies performed in rats. It was found that 40-50% of intestinally administered **genistein** appears in bile over a 4 hr period (this is also evidence of **genistein** undergoing and enterohepatic circulation). NaTC is a naturally occurring bile salt and may have a role in the absorption of **genistein** released from food.

DETD . . . on the data of the present inventors and the foregoing information, it is contemplated that to achieve a sufficiently high **genistein** concentration at the osteoclast (i.e., one capable of exerting an inhibitory effect on osteoclast activity and bone resorption) a daily intake of between about 2-50 mg and about 20-50 mg of **genistein** would be required. The difference in these figures is due to the range of inhibitory effects to be exerted on. . .

DETD . . . would be closely monitored and adjusted by an attending physician. One would particularly take note of the patient's body weight, **genistein** uptake, **genistein** secretion, age and other factors, such as, e.g., liver function, that are well known to those that conduct clinical trials and/or human treatment studies. In clinical use, the serum **genistein** levels would be monitored, as would bone density and bone turnover.

DETD Daily doses of between about 2-50 mg, or 20-50 mg, of **genistein** would be provided by 2-40 or 20-40 g of isolated soy protein, or 2-40, or 20-40 g, of other soy. . . low-fat soy milk, and a low-fat tofu (MORI-NU.TM.). Other preferred products are contemplated to be tablets comprising purified or synthetic **genistein**.

DETD . . . per day (irrespective of age). This is 4-5 g of soy protein per day, or 4-6 mg per day of **genistein** (per 300 g adult rat). Although this cannot be extrapolated to humans in a linear manner, it can be seen that daily intakes of 20-50 mg of **genistein**, as provided by 20-50 g of isolated soy protein, are perfectly reasonable quantities (based upon an average human male being 70 kg). This is supported by known daily intakes of **genistein**, which are about 70 mg in certain Asian cultures (Soybean Utilization, Eds. Snyder & Kwon, p.220). Calculations of **genistein** doses for use in other animals would be straightforward based upon the weight of the animal and the data presented. . .

DETD **GENISTEIN** CONTENT OF SOY MATERIALS

DETD The present example describes the extraction of **isoflavones** from soy products. Additional information may be found in Coward et al. (1993), incorporated herein by reference. This protocol is. . .

DETD **Isoflavones** in solid foods (analyzed in triplicate), to which 1.25 mg of fluorescein was added as an internal standard, were extracted. . . for 2 min in an Eppendorf microfuge just prior to analysis by HPLC. Tables I, II and III show the **isoflavone** concentrations of soy materials, products and foods.

DETD TABLE I

Isoflavone Concentrations.sup.a in Asian Primary Soy Materials

food	basis	conjugated genistin	conjugated daidzin	aglucones **genistein**	aglucones **daidzein**	aglucones, % total	D/G ratio **genistein**	D/G ratio **daidzein**
soy milk	g	0.130 .+-. 0.004	0.103 .+-. 0.006	0.007 .+-. 0.000	0.011 .+-. 0.002	0.252 .+-. 0.012		
	g dry wt							
. . .								

DETD TABLE II

Isoflavone Concentrations.sup.a in Processed or Fermented Asian Soy Products

soy product	basis	conjugated genistin	conjugated daidzin	aglucones **genistein**	aglucones **daidzein**	D/G aglucones, % total	D/G ratio **genistein**	D/G ratio **daidzein**
tempeh	g	0.113 .+-. 0.028	0.040 .+-. 0.013	0.164 .+-. 0.004	0.113 .+-. 0.007	0.430 .+-. 0.005		
	g dry wt	0.296.	. .					

DETD TABLE III

Isoflavone Concentrations.sup.a in Other Soy Foods

soy food	basis	conjugated genistin	conjugated daidzin	aglucones **genistein**	aglucones **daidzein**	aglucones, % total	D/G ratio **genistein**	D/G ratio **daidzein**
soy sauce	g	nd	nd	0.009 .+-. 0.002	0.014 .+-. 0.001	0.023 .+-. 0.003		
	g dry wt	nd	nd	0.036 .+-..	. .			

DETD CONDITIONS FOR EXTRACTING **GENISTEIN** FROM SOY PRODUCTS

DETD The present example indicates that, in the preparation of

isoflavones from soy products, extraction with 80% aqueous methanol at room temperature was just as efficient as extraction at 60.degree.-80.degree. C. It also shows that heated extraction causes changes in **isoflavone** composition and that heating should be avoided.

DETD 2. Extraction of **isoflavones** from soy foods

DETD **Daidzein** and **genistein** and their .beta.-glucoside conjugates were isolated and purified as described above in Example V, and in Coward et al. (1993);. . .

DETD Reversed-phase HPLC analysis of **isoflavones** was carried out on a 25 cm.times.4.6 mm Aquapore C.sub.8 column (Applied Biosystems, Foster City, Calif.). Elution was carried out. . . min, followed by 100% solvent B for 5 min. The column was equilibrated in solvent A prior to chromatography. Eluted **isoflavones** were detected by their absorbance at 262 nm. Quantitative data for **daidzein**, daidzin, **genistein** and genistin were obtained by comparison to known standards.

DETD As the molar extinction coefficients of the **daidzein** and **genistein** 6OMalGlc conjugates approximate to those of daidzin and genistin, respectively (Kudou et al., 1991), the concentrations of the 6OMalGlc and. . . the 6OAcGlc conjugates were calculated from standard curves for the corresponding .beta.-glucoside. Similarly, concentrations of glycitein were calculated from the **daidzein** standard curve, and the concentrations of the glycitein 6OMalGlc and the 6OAcGlc conjugates from the daidzin standard curve.

DETD . . . III triple quadrupole mass spectrometer (PE-Sciex, Thornhill, Ontario, Canada) equipped with two Macintosh Quadra 950 computers for data analysis. The **isoflavones** in the soy extracts were separated by reversed-phase HPLC on a 10 cm.times.4.6 mm Aquapore C.sub.8 column at a flow. . .

DETD **Isoflavone** conjugates were also separated by reversed-phase HPLC on a 10 cm.times.2.1 mm Aquapore C.sub.8 column at a flow rate of. . .

DETD Maximum recovery of the **isoflavones** from soy milk and the isolated soy protein with 80% aqueous methanol'sufficient for reproducible quantitative measurements was obtained by tumbling. . . 2 hours (Tables IV and V). Furthermore, for both soy products, there were no significant differences in overall recovery of **isoflavones** when extraction was performed at room temperature as opposed to 60.degree. C. (Tables IV and V). Coefficients of variation obtained using the method described declined as the total **isoflavone** content rose (5.8%, 2.8%, and 1.6% for soy milk 2, soy milk 1 and isolated soy protein, respectively). In addition,. . . 80% aqueous methanol and 80% aqueous acetonitrile containing 0.1% HCl for the 2 h room temperature extraction of the total **isoflavones** in toasted soy flour.

DETD In the case of the soy milks, the **isoflavones** were present in the extracts almost entirely as their .beta.-glucoside conjugates whether extracted at room temperature or at 60.degree. C. In contrast, for the isolated soy protein, although extraction at 60.degree. C. did not change the total **isoflavone** concentration, it did cause significant changes in the composition of the **isoflavone** conjugates. At each time point in the extraction, genistin and **genistein** concentrations were increased at the expense of **genistein** 6-OMalGlc (Table V). Similar trends were also observed for daidzin, **daidzein** and **daidzein** 6-OMalGlc. When the extraction temperature was increased to 80.degree. C., the conversion of the **isoflavone** 6-OMalGlc conjugates to the .beta.-glucoside conjugates was much greater and was time-dependent (FIG. 5). Heated extraction of toasted soy flour at 80.degree. C. in 80% aqueous methanol also led to conversion of **isoflavone** 6-OAcGlc conjugates to their .beta.-glucoside conjugates (FIG. 6). Even when kept at room temperature, **isoflavones** in 80% aqueous methanol extracts of the soy materials were converted gradually from the

6-OMalGlc forms to the .beta.-glucosides.

DETD . . . use of acetic acid in the HPLC mobile phase did not lead to a significant improvement in resolution of the **isoflavone** conjugates compared to trifluoroacetic acid. When ammonium acetate was included in the aqueous buffer (as used for analysis by HPLC-MS), the elution volumes of the **isoflavone** 6-OMalGlc conjugates decreased sharply due to ionization of their carboxyl groups.

DETD TABLE IV

Effect of mixing time and heat on the extraction of **isoflavones** (.mu.g/gm)* from soy milks

	Room temperature			60.degree. C.		
	1 h	2 h	24 h	1 h	2 h	4 h
Soy milk #1						
Daidzin	89.4 .+-. 3.2		87.0 .+-. 0.9	83.7 .+-. 4.8	86.2 .+-. 1.4	87.2 .+-. 0.3	88.6 .+-. 1.1
Daidzein	3.7 .+-. 0.4		3.3 .+-. 0.4	2.6 .+-. 0.2	3.8 .+-. 1.1	3.1 .+-. 0.4	3.0 .+-. 0.4
Genistin	143 .+-. 2.6		138 .+-. 1.9	135 .+-. 6.3	140 .+-. 2.0	138 .+-. 1.4	139 .+-. 0.4
Genistein	35.5 .+-. 1.6		35.1 .+-. 1.6	31.9 .+-. 1.8	31.4 .+-. 1.5	30.9 .+-.	

1.5
29.1 .+-.
1.4
6-OMalGlc
Genistein
3.4 .+-.
0.8
3.6 .+-.
0.4
2.8 .+-.
0.3
5.4 .+-.
1.6
3.2 .+-.
0.4
3.1 .+-.
0.6
Glycitin
7.9 .+-.
. . . 3
Soy milk #2
Daidzin
52.2 .+-.
2.9
53.2 .+-.
4.4
52.4 .+-.
0.9
50.2 .+-.
0.8
50.8 .+-.
2.1
50.3 .+-.
2.6
Daidzein
2.1 .+-.
0.9
1.2 .+-.
0.9
.9 .+-.
0.8
1.6 .+-.
0.9
0.7 .+-.
0.9
n.d.
Genistin
80.2 .+-.
2.6
81.0 .+-.
4.8
80.1 .+-.
0.6
78.4 .+-.
1.9
78.9 .+-.
1.6
82.9 .+-.
5.7
Genistein
12.3 .+-.
1.4
13.5 .+-.
3.5

12.4 .+-.
2.8
10.8 .+-.
0.3
14.1 .+-.
3.2
13.0 .+-.
0.9

6-OMalGlc

Genistein

1.5 .+-.
0.6
1.5 .+-.
0.3
1.3 .+-.
0.2
0.8 .+-.
0.6
1.9 .+-.
0.5
1.2 .+-.
0.1

Glycitin

9.6 .+-.

. . .

DETD TABLE V

Effect of mixing time and heating to 60.degree. C. on the extraction of **isoflavones** (.mu.g/gm)* from a soy protein isolate

	Room temperature			60.degree. C.		
Isoflavone	1 h	2 h	24 h	1 h	2 h	4 h
Daidzin	275 .+-. 6	272 .+-. 9	297 .+-. 5	297 .+-. 2.sup.a	309 .+-. 8.sup.b	326 .+-. 2.sup.c
Daidzein	374 .+-. 9	361 .+-. 6	357 .+-. 4	344 .+-. 4.sup.a	328 .+-. 5.sup.b	318 .+-. 8.sup.c
6-OMalGlc **Daidzein**	93 .+-. 5	95 .+-. 4	92 .+-. 1	94 .+-. 3	93 .+-. 4	93 .+-. 2
6-OAcGlc						

Daidzein
52 .+-.
4 59 .+-.
5 57 .+-.
2 56 .+-.
11
66 .+-.
4 64 .+-.
4

Genistin
462 .+-.
9 460 .+-.
16
503 .+-.
10
512 .+-.
4.sup.a
536 .+-.
7.sup.b
571 .+-.
5.sup.c

Genistein
752 .+-.
16
762 .+-.
4 735 .+-.
17
707 .+-.
6.sup.a
684 .+-.
13.sup.b
654 .+-.
15.sup.c

6-OMalGlc
Genistein
141 .+-.
8 142 .+-.
5 142 .+-.
4 140 .+-.
1 143 .+-.
6 148 .+-.
4

6-OAcGlc
Genistein
49 .+-.
6 46 .+-.
9 43 .+-.
3 56 .+-.
8.sup.a
59 .+-.
3.sup.b
55 .+-.
8.sup.c

Glycitin
86 .+-.
. . .

DETD When analyzed by HPLC-HN-APCI mass spectrometry in the positive ion mode, three different types of conjugates were identified for each **isoflavone**. In the case of defatted soy flour (FIG. 7) and soybean hypocotyls (FIG. 8), the principal conjugate was the 6-OMalGlc of each of the three **isoflavones**, **daidzein** (7,4'-dihydroxyisoflavone), **genistein** and glycitein (7,4'-dihydroxy-6-methoxyisoflavone). In contrast, in soy molasses and tofu, the principal conjugates were the **isoflavone** bglucosides

(FIG. 8). Toasted soy flour and the isolated soy protein contained large amounts of the 6-OAcGlc conjugates (FIG. 7).

DETD **Isoflavone** 6-OMalGlc conjugates The major ions in the HN-APCI positive ion mass spectrum of each **isoflavone** 6-OMalGlc conjugate when analyzed by HPLC in a background of 0.1% acetic acid (FIG. 9) were the [M+H].sup.+ molecular ion,. . .

DETD . . . whether analyzed by HPLC in 0.1% acetic acid or in 10 mM ammonium acetate, the molecular [M-H].sup.- ion for each **isoflavone** 6-OMalGlc was not observed. Instead, the [M-MalGlc-H].sup.- aglucone ion was the principal ion. The other major ions were the [M-COOH]-. . .

DETD Positive ion mass spectra for each **isoflavone** 6-OMalGlc obtained with the IonSpray.TM. interface showed that under these conditions (no heating) the intact [M+H].sup.+ molecular ion was the. . .

DETD **Isoflavone** b-glucoside conjugates Daidzin, genistin and glycitin gave rise to two main ions using the HN-APCI interface, the molecular [M+H].sup.+ ion. . .

DETD **Isoflavone** 6-OAcGlc conjugates These conjugates were the least abundant of the conjugates in all soy fractions tested although significant amounts were. . . aglucone ion [M+H-AcGlc].sup.+, whereas in negative ion spectra only the acetate adduct [M-H+CH.sub.3 COO].sup.- was observed. In the case of **genistein** 6-OAcGlc, the acetate adduct ion was the most abundant ion.

DETD The relative sensitivity of HN-APCI and IonSpray.TM. for the detection of **isoflavone** glycosidic conjugates by HPLC-MS was assessed using a 80% aqueous methanol extract of toasted soy flour. The highest sensitivity for each type of conjugate was observed for the positive **isoflavone** aglucone ions (m/z values, 255, 271 and 285) generated in the HN-APCI interface. They were mostly 1.5 to 3-fold more. . .

DETD 3. **Isoflavone** conjugate intestinal hydrolysis and absorption

DETD The inventors also found that the chemical form of **genistein** will affect the sites of absorption from the intestines. Unconjugated **genistein** is fully protonated under the acid conditions of the stomach and would be transported by passive diffusion from the lumen. . .

DETD The conjugated forms of **genistein** are not absorbed by passive absorption and therefore must undergo hydrolysis to release the unconjugated **genistein**. Their hydrolysis does not occur under the acid conditions of the stomach, but instead requires the action of specific enzymes.

DETD . . . the .beta.-glucosidase activity of the small intestine is sufficient for substantial hydrolysis to occur there, leading to the appearance of **isoflavones** in the blood which peak at 2 hours. This is consistent with absorption occurring in the small intestine. The inventors. . .

DETD . . . inventors have found that when volunteers (5) consumed a soy protein beverage (Supro) rich in the 6"-O-acetylglucosides and the 6"-O-malonylglucosides, **isoflavones** did not appear in the blood and urine for 4-8 hours afterwards (unlike full-fat soy milk where blood levels peaked. . . at 2 hours after ingestion). Finally, the search for tyrosine kinase inhibitors formed by microorganisms led to the discovery of **genistein**. **Genistein** was not synthesized by the microorganisms, but rather released by hydrolysis of the 6"-O-malonylglucosides in the soymeal used as a source of protein for the growth of the microorganisms. The organisms associated with the appearance of **genistein** in the growth media were those normally found in the large bowel.

DETD As part of their enterohepatic circulation, **isoflavones** are glucuronidated. The **isoflavone** glucuronides, as for other glucuronides, are hydrolyzed by .beta.-glucuronidases in the large bowel, and the unconjugated forms reabsorbed. In this. . .

DETD In the present example, it has been shown that the extraction of

isoflavone conjugates from the soy matrices tested occurs readily at room temperature in 80% aqueous methanol, being essentially complete within 1-2 hr. Heating, as used by many previous investigators, is unnecessary and, as noted above, alters the **isoflavone** composition. However at 60.degree. C., the magnitude of the heat-induced changes was considerably less than at 80.degree. C.

DETD . . . temperature. When extraction was carried out with 80% aqueous methanol at 80.degree. C. for 1-4 h, the concentration of the **isoflavone** 6-OMalGlc conjugates and 6OAcGlc conjugates (in toasted soy flour) declined as the .beta.-glucoside conjugates rose. The de-esterification reaction was presumably. . . or acetate carboxyl group and the 6"-hydroxyl group of the glucose moiety, yielding methyl malonate or methyl acetate and the **isoflavone** .beta.-glucoside. This effect may explain the apparently lower concentrations of **isoflavone** 6-OAcGlc conjugates in 80% aqueous methanol extracts of toasted soy flour, as reported previously (Farmakalidis & Murphy, 1985). It should. . . extracts for extended periods even at room temperature would be expected to lead to gradual changes in the composition of **isoflavone** conjugates.

DETD This example also shows that an important source of the observed variation in **isoflavone** conjugate composition of different soy foods is the degree of heating the soy material is exposed to during its preparation. **Isoflavone** 6-OMalGlc conjugates are prone to both heat-induced decarboxylation (to form 6-OAcGlc conjugates) and de-esterification (to form .beta.-glucoside conjugates) (Kudou et. . . tofu essentially complete de-esterification to the .beta.-glucosides was the principal chemical change. Indeed, the predominance of the .beta.-glucosides over other **isoflavone** conjugates in soy molasses has enabled investigators to isolate genistin (the .beta.-glucoside of **genistein**) on a large scale from this matrix (Barnes et al., 1994; Coward et al., 1993; Walter, 1941).

DETD Since the **isoflavone** 6-OAcGlc conjugates are virtually absent from extracts of the soybean cotyledon and hypocotyl, but are present in large quantities in. . .

DETD . . . also describes for the first time the application of HPLC-mass spectrometry with HN-APCI and IonSpray.TM. interfaces to the analysis of **isoflavones**. **Isoflavones** in soy foods are more readily detected using the aglucone ions generated in the HN-APCI interface than molecular ions or. . .

DETD The data confirm findings that, in addition to the .beta.-glucoside conjugates, **isoflavones** in soy hypocotyl and cotyledon are also present as 6-OMalGlc conjugates (Kudou et al., 1991) and in toasted soy flour. . .

DETD The **isoflavone** b-glucosides and 6-OAcGlc conjugates yielded molecular ions in both positive and negative ion mass spectra, but the most abundant ion in each case was the aglucone ion. Therefore, to identify the conjugates of an **isoflavone** in serial mass spectra obtained following reversed-phase HPLC separation of food extracts, selected ion chromatograms were prepared with a combination. . . for conjugates of glycitein since their mass spectra contained a much lower relative abundance of the molecular ion compared with **daidzein** and **genistein** conjugates.

DETD Introduction of **isoflavone** .beta.-glucoside and 6-OAcGlc ions into the mass spectrometer via the IonSpray.TM. interface resulted in a lower sensitivity than using the. . .

DETD . . . reduced by increasing the orifice potential. The relative molar ion yields for the three types of conjugates and the three **isoflavones** varied only over a two fold range and were similar in positive and negative ion mass spectra.

DETD The inventors also found that the 6"-O-substitution has an effect on the susceptibility of the **isoflavone** conjugates to intestinal hydrolysis and hence absorption. There are thus differences in bioavailability and metabolism of the **isoflavones** dependent on the nature of their chemical form.

DETD Adlercreutz; International Symposium on **Phytoestrogens**; Little Rock, Ark., Oct. 17-20th, 1993; Proceedings published in Proc. Soc. Exptl. Biol. Med.

DETD Setchell; International Symposium on **Phytoestrogens**; Little Rock, Ark., Oct. 17-20th, 1993; Proceedings published in Proc. Soc. Exptl. Biol. Med.

CLM What is claimed is:

. . . A method for reducing acid secretion by osteoclasts, comprising contacting an osteoclast with a composition comprising an amount of a **genistein**-glucoside conjugate that occurs naturally in soy, the amount effective to inhibit osteoclast acid secretion.

2. The method of claim 1, wherein the **genistein** conjugate is a 6-O"-acetylglucoside conjugate or a 6-O"-malonylglucoside conjugate.

3. The method of claim 1, wherein the **genistein** conjugate is obtained from soy or a soy product.

4. The method of claim 1, wherein the **genistein** conjugate is a synthetic form of a **genistein** conjugate that occurs naturally in soy.

5. The method of claim 1, wherein the osteoclast is located within an animal and the **genistein** conjugate is administered to said animal in a pharmaceutically acceptable form.

6. The method of claim 5, wherein the **genistein** conjugate is administered to said animal in the form of a tablet.

7. The method of claim 5, wherein the **genistein** conjugate is administered to said animal in the form of a soy food product.

8. The method of claim 7, wherein the **genistein** conjugate is administered to said animal in the form of an unprocessed soy food or isolated soy protein composition.

. . . comprising administering to an animal exhibiting symptoms associated with bone resorption an amount of a pharmaceutically acceptable composition comprising a **genistein**-glucoside conjugate that occurs naturally in soy, the amount effective to reduce acid secretion by osteoclasts located within the animal.

10. The method of claim 9, wherein the pharmaceutically acceptable composition comprises a 6-O"-acetylglucoside or 6-O"-malonylglucoside **genistein** conjugate.

11. The method of claim 9, wherein the pharmaceutically acceptable **genistein** conjugate composition is administered orally.

12. The method of claim 9, wherein the pharmaceutically acceptable **genistein** conjugate composition is in the form of a tablet.

13. The method of claim 9, wherein the pharmaceutically acceptable **genistein** conjugate composition is in the form of a soy food product.

. . . for treating osteoporosis, comprising administering to a patient suspected of having osteoporosis a pharmacologically effective amount of a pharmaceutically acceptable **genistein**-glucoside conjugate composition that occurs naturally in soy.

16. The method of claim 15, wherein the pharmaceutically acceptable **genistein** conjugate composition comprises a 6-O"-acetylglucoside or 6-O"-malonylglucoside **genistein** conjugate.

17. The method of claim 16, wherein the pharmaceutically acceptable **genistein** conjugate composition is in tablet form.

18. The method of claim 17, wherein the pharmaceutically acceptable **genistein** conjugate composition is a tablet comprising 2-50 mg of the **genistein** conjugate.

19. The method of claim 18, wherein the pharmaceutically acceptable **genistein** conjugate composition is a tablet comprising 5-50 mg of the **genistein** conjugate.

20. The method of claim 19, wherein the pharmaceutically acceptable **genistein** conjugate composition is a tablet comprising 10-50 mg of the **genistein** conjugate.

21. The method of claim 20, wherein the pharmaceutically acceptable **genistein** conjugate composition is a tablet comprising 20-50 mg of the **genistein** conjugate.

22. The method of claim 21, wherein the pharmaceutically acceptable **genistein** conjugate composition is a tablet comprising 50 mg of the **genistein** conjugate.

23. The method of claim 16, wherein the pharmaceutically acceptable **genistein** conjugate composition is a soy food product.

24. The method of claim 23, wherein the pharmaceutically acceptable **genistein** conjugate composition is 2-50 mg of isolated soy protein or unprocessed soy food.

25. The method of claim 24, wherein the pharmaceutically acceptable **genistein** conjugate composition is 5-50 mg of isolated soy protein or unprocessed soy food.

26. The-method of claim 25, wherein the pharmaceutically acceptable **genistein** conjugate composition is 10-50 mg of isolated soy protein or unprocessed soy food.

27. The method of claim 26, wherein the pharmaceutically acceptable **genistein** conjugate composition is 20-50 mg of isolated soy protein or unprocessed soy food.

L3 ANSWER 540 OF 540 USPATFULL on STN
ACCESSION NUMBER: 95:52376 USPATFULL
TITLE: Pharmaceutical compositions and dietary soybean food products for the prevention of osteoporosis
INVENTOR(S): Shlyankevich, Mark, Waterbury, CT, United States
PATENT ASSIGNEE(S): Bio-Virus Research Incorporated, San Matteo, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5424331		19950613	
APPLICATION INFO.:	US 1994-258460		19940610	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Hollinden, Gary E.
ASSISTANT EXAMINER: Burn, Brian M.
LEGAL REPRESENTATIVE: Dubno, Herbert, Myers, Johathan
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
LINE COUNT: 391

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB (a) 75 to 200 parts of one or more **phytoestrogen** compounds;

SUMM . . . prevention of osteoporosis. More particularly, the invention relates to such pharmaceutical compositions and dietary soybean food products that contain natural **phytoestrogens** of the **isoflavone** or coumestan groups.

SUMM . . . amounts of soy products. According to The Lancet, Vol. 339, p. 1233 (16 May 1992), H. Adlercreutz et al, Dietary **Phytoestrogens** and the Menopause in Japan, the diet high in soy may be the reason why menopausal symptoms are much less. . . of several Japanese men, women, and children was analyzed and the urine was found to contain a high amount of **phytoestrogens**. There is no mention or suggestion in this reference, however, of specifically avoiding osteoporosis thanks to a high soy diet.

SUMM The **phytoestrogens** are diphenolic plant compounds that are somewhat related structurally to the mammalian sex hormone: 17-beta-estradiol. See Setchell, K. D. R., et al Am. J. Clin. Nutr., 40:569 to 578 (1984). Two chemical classes of **phytoestrogens** are abundant in soybeans, total soy products, and soy protein isolates. Those two classes are coumestrol and **isoflavones**. The latter class includes **daidzein, genistein**, glycitein, as well as their glycoside and acetylated forms. The level of **phytoestrogens** in total soybeans and their bioavailability are relatively high, and their metabolism is similar to that of endogenous sex hormones.. . .

SUMM **Phytoestrogens** and their metabolites interact with specific cell receptors and compete with endogenous hormone molecules [see Folman, Y. et al, J.. . .

SUMM **Phytoestrogens** can induce two different effects in an organism. When the level of endogenous sex hormones is relatively high, the antiestrogenic effect prevails. There are several mechanisms of antiestrogenic activity of **phytoestrogens**, including feedback inhibition at the hypothalamus and pituitary glands, and competition and blockade of cell receptors. It has been observed that a **phytoestrogen**- and lignan-rich diet is associated with the reduction of free plasma estradiol, and the risk of breast cancer. See Adlercreutz,. . . to 1144 (1987) and Mousavi, Y. et al, Steroids, 58:301 to 304 (1993). On the other hand in postmenopausal women, **phytoestrogens** can provoke an estrogenic response. See Adlercruetz, H. et al, Lancet, 339:1233 (1992). This dual effect of weak estrogens is. . .

SUMM (a) 75 to 200 parts of one or more **phytoestrogen** compounds;

SUMM The amount of **phytoestrogens** (e.g. **isoflavones**) administered per day may be 200 mg which is a preferred daily dosage of the abovementioned compositions corresponds to the amount of **isoflavones** naturally occurring in 50-75 g of raw soybeans. This is the average amount of soybeans consumed daily in an Oriental diet. 200 mg of **isoflavones** are functionally equivalent to the daily dosage of conjugated steroidal estrogen used in hormone replacement therapy.

SUMM The weak estrogenecity of soybean **phytoestrogens** and their metabolites is beneficial for saving bone mass, and for prevention of osteoporosis and fractures.

SUMM There are two classes of **phytoestrogens** that are especially contemplated to be within the scope of the present invention. Those two classes are the **isoflavones** and the coumestans. Examples of the **isoflavones** include **daidzein, genistein** , glycitein, and their glycosides: daidzin, genistin, and glycitin, as well as acetylated forms of the abovementioned compounds. An example of. . .

SUMM The new compositions according to the invention may contain any one or several **phytoestrogens** in combination. A preferred combination of the **phytoestrogens** includes: daidzin 120 to 180 parts by weight, genistin 280 to 350 parts by weight, **daidzein** 80 to

120 parts by weight and **genistein** 8 to 12 parts by weight. As a possible variant, the combination can include daidzin and genistin in equimolar concentrations,. . .

SUMM . . . to 20 mg of active ingredients per kg of body weight. By active ingredients, I do not mean only the **phytoestrogens**, but the other ingredients mentioned in the compositions as well. The optimum dosage of course depends on the body weight. . . of the osteoporosis. Such a daily dosage of the compositions will give the subject the needed volume of calcium and **phytoestrogen** necessary to improve hormonal status and to save bone mass as well as prevent fractures.

SUMM . . . a day, each containing 150 to 400 mg of the total active ingredients or 50 to 70 mg of the **phytoestrogens**.

SUMM . . . different for middle age and older adults. The USA RDA recommends higher levels of some vitamins and minerals. Natural dietary **phytoestrogens** can replace estrogen therapy. The presently disclosed compositions which contain **phytoestrogens** have the ability to improve hormonal balance, and are highly effective in the treatment of osteoporosis in older women and. . .

DETD

(1)	soybean **isoflavone** (**phytoestrogens**)	75 mg
	genistin	
(2)	licorice root extract (dried)	50 mg
(3)	calcium carbonate	750 mg
(4)	magnesium oxide	160 mg
(5)	zinc sulfate	15. . .

DETD . . . Example 1 except that the 75 mg of genistin is replaced by the 75 mg of the following mixture of **phytoestrogens**:

DETD

daidzin	160 parts
genistin	315 parts
daidzein	97 parts
genistein	9.9 parts.

DETD

soybean coumestrin (**phytoestrogen**) coumestrol	50 mg
calcium carbonate	300 mg
magnesium oxide	160 mg
zinc sulfate	25 mg
beta-carotene	20 mg
Vitamin D (as cholecalciferol)	5 mcg

Vitamin E. . .

CLM What is claimed is:

. . . A composition for the treatment or prevention of osteoporosis which comprises: (a) 75 to 200 parts of one or more **phytoestrogen** compounds, wherein the **phytoestrogen** compound is a coumestan or an **isoflavone** selected from the group consisting of **daidzein**, **genistein**, glycitein, daidzin, genistin, glycitin, an acetylated form thereof and mixtures thereof; (b) 0 to 100 parts of dried licorice root. . .

2. The composition for the treatment or prevention of osteoporosis defined in claim 1 wherein the **phytoestrogen** compound is a coumestan.

3. The composition for the treatment or prevention of osteoporosis defined in claim 1 wherein the mixture of **isoflavones** includes: 120 to 180 parts by weight of daidzin; 280 to 350 parts of genistin; 80 to 120 parts by weight of **daidzein**; and 8 to 12 parts by weight of **genistein**,

L3 ANSWER 202 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:556053 CAPLUS
DOCUMENT NUMBER: 121:156053
TITLE: Quantitation of **Phytoestrogens** in Legumes by HPLC
AUTHOR(S): Franke, Adrian A.; Custer, Laurie J.; Cerna, Carmencita M.; Narala, Kavitha K.
CORPORATE SOURCE: Cancer Research Center of Hawaii, Honolulu, HI, 96813, USA
SOURCE: Journal of Agricultural and Food Chemistry (1994), 42(9), 1905-13
CODEN: JAFCAU; ISSN: 0021-8561
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Quantitation of **Phytoestrogens** in Legumes by HPLC
AB A fast, sensitive, and precise method is presented for the efficient extn. and quantitation of coumestrol, **daidzein**, **genistein**, formononetin, and biochanin A from foods by diode array reversed-phase HPLC anal. using flavone as internal std. Acid hydrolysis during extn. of foods was chosen to convert the various **phytoestrogen** conjugates into their resp. aglycons, facilitating HPLC anal. and allowing quantitation of total **phytoestrogens** as aglycons including originally present glycosides, "free" aglycons, and those conjugates which are below the detection limit in food plants. Extn. efficiencies and HPLC conditions were evaluated and optimized, leading to precision and spiking recovery values of 3-8% and 94-104%, resp., depending on the analyte. **Phytoestrogen** levels from more than 40 food items, mostly legumes, were detd. using this method. High levels of **daidzein** and **genistein** were found in soy products and black beans, whereas sprout items were rich in coumestrol and formononetin.
ST estrogen detn legume HPLC; chromatog liq coumestrol **isoflavone** **phytoestrogen**
IT Legume
(coumestrol and estrogenic **isoflavone** detn. in, by HPLC)
IT Vigna radiata
(**phytoestrogens** detn. in seeds and sprouts of, by HPLC)
IT Pea
(**phytoestrogens** detn. in split, by HPLC)
IT Clover
(**phytoestrogens** detn. in sprouts of, by HPLC)
IT Alfalfa
Bean
Black bean
Broad bean
Chickpea
Food analysis
Red bean
Soybean
Soybean curd
(**phytoestrogens** detn. in, by HPLC)
IT Bean
(P. limensis, **phytoestrogens** detn. in, by HPLC)
IT Soybean
(flour, **phytoestrogens** detn. in, by HPLC)
IT Chromatography, column and liquid
(high-performance, of **phytoestrogens** in legumes)
IT Bean
(kidney, **phytoestrogens** detn. in, by HPLC)
IT Bean
(navy, **phytoestrogens** detn. in, by HPLC)
IT Bean
(pinto, **phytoestrogens** detn. in, by HPLC)
IT Bean

(white, **phytoestrogens** detn. in, by HPLC)
IT 446-72-0, **Genistein** 479-13-0, Coumestrol 485-72-3, Formononetin 486-66-8, **Daidzein** 491-80-5, Biochanin A
RL: ANT (Analyte); ANST (Analytical study)
(detn. of, in legumes by HPLC)

L3 ANSWER 203 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:296985 CAPLUS
DOCUMENT NUMBER: 120:296985
TITLE: Study of the **phytoestrogen** content of goat's rue (Galega orientalis), alfalfa (Medicago sativa) and white clover (Trifolium repens)
AUTHOR(S): Saloniemi, Hannu; Kallela, Kaarlo; Saastamoinen, Ilkka
CORPORATE SOURCE: Dept. Basic Vet. Med., Coll. Vet. Med., Helsinki, 00581, Finland
SOURCE: Agricultural Science in Finland (1993), 2(6), 517-24
CODEN: ASFIEB; ISSN: 0789-600X
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Study of the **phytoestrogen** content of goat's rue (Galega orientalis), alfalfa (Medicago sativa) and white clover (Trifolium repens)
AB Studies were conducted to det. the **phytoestrogen** content of goat's rue (Galega orientalis Lam.), alfalfa (Medicago sativa L.) and white clover (Trifolium repens L.), all belonging to the Fabaceae and subjected to test cultivation at research stations of the Agricultural Research Center of Finland. Apart from some insignificant quantities, goat's rue did not contain any known **phytoestrogens**. Even in biol. studies it had no estrogenic effect. The estrogenic effect of alfalfa was apparently due to coumestrol, which was discovered in the samples in quantities of 34-65 ppm. All white clover varieties contained very small quantities of estrogenic **isoflavones** and coumestrol, and they did not explain the increased wt. of the immature rat uterus obsd. in the biol. studies.
ST **phytoestrogen** Galega alfalfa clover; uterus wt fodder legume **phytoestrogen**
IT Alfalfa
Galega orientalis
(**phytoestrogens** of, fodder quality in relation to)
IT Uterus
(wt. of, dietary alfalfa and white clover effect on, **phytoestrogen** content in relation to)
IT Clover
(T. repens, **phytoestrogens** of, fodder quality in relation to)
IT 486-66-8, **Daidzein** 491-80-5, Biochanin A
RL: ANT (Analyte); ANST (Analytical study)
(detn. of, in alfalfa and white clover by HPLC, hydrolysis method in relation to)
IT 446-72-0, **Genistein** 479-13-0, Coumestrol 485-72-3, Formononetin
RL: OCCU (Occurrence)
(of alfalfa and white clover, fodder quality in relation to)

L3 ANSWER 204 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:62290 CAPLUS
DOCUMENT NUMBER: 120:62290
TITLE: Health supplements containing **phytoestrogens**, analogs, or metabolites thereof
INVENTOR(S): Kelly, Graham Edmund
PATENT ASSIGNEE(S): Australia
SOURCE: PCT Int. Appl., 28 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9323069	A1	19931125	WO 1993-AU230	19930519
W: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9340525	A1	19931213	AU 1993-40525	19930519
AU 683838	B2	19971127		
EP 656786	A1	19950614	EP 1993-909679	19930519
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 07506822	T2	19950727	JP 1993-519718	19930519
NO 9404435	A	19941118	NO 1994-4435	19941118
US 5830887	A	19981103	US 1995-338567	19950112
US 6562380	B1	20030513	US 1997-910837	19970813
US 6642212	B1	20031104	US 1999-421069	19991019
US 2003078214	A1	20030424	US 2002-274371	20021021

PRIORITY APPLN. INFO.:		
AU 1992-2511	A	19920519
WO 1993-AU230	A	19930519
US 1995-338567	A1	19950112
US 1997-910837	A1	19970813
US 2000-547100	A1	20000411

TI Health supplements containing **phytoestrogens**, analogs, or metabolites thereof

AB Compns. enriched with natural **phytoestrogens** or analogs thereof selected from **genistein**, **daidzein**, formononetin, and biochanin A are used as food additives, tablets or capsules for promoting health in cases of cancer, premenstrual syndrome, menopause or hypercholesterolemia. Thus, dried red clover was extd. with a solvent mixt. contg. water, alc., $CHCl_3$, acetone, and/or EtOAc. Cholesterol-lowering effects of the obtained **isoflavones** were demonstrated with normal individuals.

ST health supplement **phytoestrogen**

IT Soybean
(**phytoestrogen** extn. from, health supplements contg.)

IT Nutrients
(**phytoestrogens** as supplements for)

IT Menopause
Neoplasm
(**phytoestrogens** for promotion of health in)

IT Clover
(T. pratense, **phytoestrogen** extn. from, health supplements contg.)

IT Pharmaceutical dosage forms
(capsules, **phytoestrogens** in, for promotion of health)

IT Mammary gland
(disease, benign, **phytoestrogens** for promotion of health in)

IT Ovarian cycle
(disorder, premenstrual syndrome, **phytoestrogens** for promotion of health in)

IT Plant organ
(hypocotyl, of soybean, **phytoestrogen** extn. from, health supplements contg.)

IT Mammary gland
(neoplasm, **phytoestrogens** for promotion of health in)

IT Pharmaceutical dosage forms
(tablets, **phytoestrogens** in, for promotion of health)

IT 446-72-0, **Genistein** 485-72-3, Formononetin 486-66-8, **Daidzein** 491-80-5, Biochanin A
RL: BIOL (Biological study)
(health supplements contg.)

IT 57-88-5, Cholesterol, biological studies
RL: BIOL (Biological study)
(metabolic disorders, hypercholesterolemia, **phytoestrogens** for promotion of health in)

L3 ANSWER 205 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1991:162581 CAPLUS
DOCUMENT NUMBER: 114:162581
TITLE: Uncoupling and inhibition of the respiratory chain in rat liver mitochondria by some naturally occurring estrogens and their metabolites
AUTHOR(S): Lundh, Torbjoern J. O.; Lundgren, Bjoern O.
CORPORATE SOURCE: Dep. Anim. Nutr. Manage., Swed. Univ. Agric. Sci., Uppsala, S-75007, Swed.
SOURCE: Journal of Agricultural and Food Chemistry (1991), 39(4), 736-9
CODEN: JAFCAU; ISSN: 0021-8561
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effects on oxidative phosphorylation in rat mitochondria of some of the most common food plant estrogenic **isoflavones** and zearalenone and their metabolites were assayed by polarog. with an O electrode. In general, the substances act as uncoupling agents. At 100 .mu.M concns., biochanin A and formononetin had 10-50% of the uncoupling activity of 2,4-dinitrophenol; coumestrol and **genistein** had lesser effects, and **daidzein** and equol caused only slight uncoupling. Zearalenone, .alpha.-zearalenol, and .beta.-zearalenol had an uncoupling activity about equal to that of **genistein**. The **isoflavones** with highest uncoupling were most effective as inhibitors of the respiratory chain and inhibited the electron transport at the same site as rotenone. The potential effect in vivo is discussed.
ST oxidative phosphorylation mitochondria **phytoestrogen** feed; **isoflavone** electron transport; zearalenone electron transport; electron transport feed **phytoestrogen**; liver mitochondria feed **phytoestrogen**; estrogen feed mitochondria phosphorylation
IT Animal respiration
(by liver mitochondria, inhibition of, by **phytoestrogens** and zearalenone and metabolites)
IT Liver, metabolism
(oxidative phosphorylation inhibition in mitochondria, by **phytoestrogens** and zearalenone and metabolites)
IT Feed
(**phytoestrogens** and zearalenone and metabolites from, mitochondrial respiration inhibition and electron transport uncoupling by)
IT Electron transport system, biological
(uncoupling of, by **phytoestrogens** and zearalenone and metabolites)
IT 446-72-0, **Genistein** 479-13-0, Coumestrol 485-72-3, Formononetin 486-66-8, **Daidzein** 491-80-5, Biochanin A 531-95-3, Equol 17924-92-4, Zearalenone 36455-72-8, .alpha.-Zearalenol 71030-11-0, .beta.-Zearalenol
RL: BIOL (Biological study)
(mitochondrial respiration inhibition and electron transport uncoupling by)

L3 ANSWER 206 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:53823 CAPLUS
DOCUMENT NUMBER: 112:53823
TITLE: A simplified HPLC method for the determination of **phytoestrogens** in soybean and its processed products
AUTHOR(S): Wang, Guangjian; Kuan, Shia S.; Francis, Octave J.; Ware, George M.; Carman, Allen S.

CORPORATE SOURCE: Nat. Toxins Res. Cent., Food and Drug Adm., New Orleans, LA, 70122, USA
SOURCE: Journal of Agricultural and Food Chemistry (1990), 38(1), 185-90
CODEN: JAFCAU; ISSN: 0021-8561
DOCUMENT TYPE: Journal
LANGUAGE: English

TI A simplified HPLC method for the determination of **phytoestrogens** in soybean and its processed products

AB **Phytoestrogens** (**daidzein**, **genistein**, coumestrol) can be isolated from soybean and its processed products (tofu, meal, yuba, sprouts, soy sauce, and beverage) and subsequently quantitated by HPLC without defatting and cleanup of the samples prior to assay. The samples are extd. with MeCN-H2O, and the ext. is filtered through a glass fiber filter. The analytes in the filtrate are in turn sepd. by HPLC on a .mu.Bondapak C18 column with MeOH-1M NH4OAc (3:2) and quantified by spectrometry. The method is sensitive to 2 ppm of **isoflavones** with UV detection and 0.5 ppm of coumestrol with fluorescent detection. The recoveries of **phytoestrogens** in spiked samples were 75-110%. The rapidity, simplicity, and low cost of the method make feasible the assay of large nos. of samples in a regulatory lab.

ST soybean estrogen detn; HPLC **phytoestrogen**; chromatog **phytoestrogen**; **phytoestrogen** detn HPLC

IT Germination
(of soybeans, **phytoestrogens** increase in)

IT Soy sauce
Soybean
Soybean curd
Soybean meal
(**phytoestrogens** detn. in, by HPLC)

IT Soybean
(milk, **phytoestrogens** detn. in, by HPLC)

IT Soybean
(milk, yuba, **phytoestrogens** detn. in, by HPLC)

IT 446-72-0, **Genistein** 479-13-0, Coumestrol 486-66-8, **Daidzein**
RL: ANT (Analyte); ANST (Analytical study)
(detn. of, in soybeans and soybean products by HPLC)

L3 ANSWER 207 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1989:152894 CAPLUS
DOCUMENT NUMBER: 110:152894
TITLE: Development and application of a high-performance liquid chromatographic method for the analysis of **phytoestrogens**
AUTHOR(S): Jones, Amanda E.; Price, Keith R.; Fenwick, G. Roger
CORPORATE SOURCE: Inst. Food Res., AFRC, Norwich, NR4 7UA, UK
SOURCE: Journal of the Science of Food and Agriculture (1989), 46(3), 357-64
CODEN: JSFAAE; ISSN: 0022-5142
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Development and application of a high-performance liquid chromatographic method for the analysis of **phytoestrogens**

AB A high performance liq. chromatog. technique was developed for the sepn. and quantification of **isoflavones** and coumestans possessing known estrogenic activity. A reversed-phase Spherisorb ODS 2 (5 .mu.m) column was used, with buffered pH 7.5 acetonitrile-H2O as mobile phase and UV detection. This system was applied to an examn. of samples from the British Total Diet Study and 4 com. available soybean-based foods.

ST **phytoestrogen** detn food HPLC; liq chromatog **phytoestrogen**; **isoflavone** detn food HPLC; coumestan detn food HPLC

IT Soybean

(food products, **phytoestrogens** detn. in, by HPLC)
IT Alfalfa
Food analysis
Silage
(**phytoestrogens** detn. in, by HPLC)
IT Chromatography, column and liquid
(high-performance, reversed-phase, of **phytoestrogens**)
IT 446-72-0, **Genistein** 479-13-0, Coumestrol 485-72-3, Formononetin 486-66-8, **Daidzein** 491-80-5, Biochanin A 529-59-9, Genistin 552-66-9, Daidzin
RL: ANT (Analyte); ANST (Analytical study)
(detn. of, in food by HPLC)

L3 ANSWER 208 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1989:51493 CAPLUS
DOCUMENT NUMBER: 110:51493
TITLE: Identification of **phytoestrogens** in the urine of male dogs
AUTHOR(S): Juniewicz, P. E.; Pallante Morell, S.; Moser, A.; Ewing, L. L.
CORPORATE SOURCE: Dep. Popul. Dyn., Johns Hopkins Sch. Hyg. Public Health, Baltimore, MD, 21205, USA
SOURCE: Journal of Steroid Biochemistry (1988), 31(6), 987-94
CODEN: JSTBBK; ISSN: 0022-4731
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Identification of **phytoestrogens** in the urine of male dogs
AB Thermospray-mass spectrometry and gas chromatog./mass spectrometry were used to identify the **phytoestrogens daidzein**, equol, formononetin, and **genistein** in HPLC purified fractions of urine obtained from male beagles. Using the same techniques the presence of **daidzein** and **genistein** was confirmed in the com. diet fed to these same dogs. Using the immature rat uterine cytosol estrogen receptor assay, relative binding affinities of 0.08, 1.1, <0.01, and 3.9% were obtained for daidzen, equol, formononetin, and **genistein**, resp. when compared to estradiol (100%). In conclusion, **phytoestrogens** are present in urine of male beagles. Moreover, the com. diet fed to these dogs contains **isoflavones** which can be converted to equol by intestinal microflora. The need for investigations of **phytoestrogens** (e.g. equol) excreted into the urine daily and its relationship to the incidence and severity of benign prostatic hyperplasia in the dog is indicated.
ST **phytoestrogen** urine dog; estrogen phyto urine dog
IT Uterus, composition
(estrogen receptors of, **phytoestrogens** of male dog urine binding of)
IT Receptors
RL: BIOL (Biological study)
(**phytoestrogen** of urine of male dog binding of, of uterus)
IT Canis familiaris
(**phytoestrogens** of urine of male)
IT Feed
(**phytoestrogens** of, for dog, urine in relation to)
IT Urine
(**phytoestrogens** of, of male dog)
IT Estrogens
RL: BIOL (Biological study)
(receptors for, of uterus, **phytoestrogens** of urine of male dog binding by)
IT 446-72-0, **Genistein** 485-72-3 486-66-8 491-80-5, Biochanin A 531-95-3, Equol
RL: BIOL (Biological study)
(of urine, of male dog)
IT 50-28-2, Estradiol, biological studies

RL: BIOL (Biological study)
(uterus receptor binding of, **phytoestrogen** of urine of male dog inhibition of)

L3 ANSWER 209 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1988:524137 CAPLUS
DOCUMENT NUMBER: 109:124137
TITLE: In vitro metabolism of formononetin and biochanin A in bovine rumen fluid
AUTHOR(S): Dickinson, J. M.; Smith, G. R.; Randel, R. D.; Pemberton, I. J.
CORPORATE SOURCE: Extension Cent., Texas A and M Univ. Agric. Res., Overton, TX, 75684, USA
SOURCE: Journal of Animal Science (Savoy, IL, United States) (1988), 66(8), 1969-73
CODEN: JANSAG; ISSN: 0021-8812
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **phytoestrogens** formononetin and biochanin A were independently incubated in vitro at 39.degree. in bovine rumen fluid from a fistulated steer receiving an alfalfa hay diet. Formononetin was incubated in studies 1 and 2, whereas biochanin A was incubated in study 3. The **isoflavones** were sepd. and quantified by HPLC. In study 1, formononetin concn., 14.80 .mu.g/mL at time 0 declined to 1.16 .mu.g/mL by 12 h and to 0.76 .mu.g/mL by 24 h. **Daidzein**, 0.18 .mu.g/mL at time 0, peaked at 12.92 .mu.g/mL at 6 h and decreased to 1.30 .mu.g/mL by 24 h. Equol, detected at 6 h, peaked at 16.94 .mu.g/mL at 18 h and dropped to 12.64 .mu.g/mL at 24 h. In incubation study 2, formononetin declined from 17.57 .mu.g/mL at time 0 to 7.08 .mu.g/mL by 6 h. **Daidzein** concn. was 1.75 .mu.g/mL at time 0 and increased to 12.03 .mu.g/mL by 6 h. Equol was detected at 3 h and increased to 2.32 .mu.g/mL at 6 h. The half-lives were 4.3 for formononetin and 9.8 h for **daidzein** in this in vitro system. In study 3, biochanin A, 8.54 .mu.g/mL at time 0, decreased to 0 .mu.g/mL by 12 h in incubation 3, whereas **genistein**, 3.17 .mu.g/mL at 1 h, peaked at 7.35 .mu.g/mL at 4 h and decreased to 0.32 .mu.g/mL at 24 h. Equol was not detected in incubation study 3. The half-lives of biochanin A and **genistein** were 3.9 and 5.5 h, resp. Bovine rumen fluid metab. of formononetin yielded **daidzein** and equol, but the only identified product of biochanin A was **genistein**.
IT 446-72-0, **Genistein**
RL: BIOL (Biological study)
(as biochanin A metabolite, of in vitro rumen fluid)

L3 ANSWER 210 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1984:471137 CAPLUS
DOCUMENT NUMBER: 101:71137
TITLE: Liquid chromatographic determination of the plant estrogens coumestrol and **isoflavones** in animal feed
AUTHOR(S): Pettersson, Hans; Kiessling, Karl Heinz
CORPORATE SOURCE: Dep. Anim. Nutr., Swed. Univ. Agric. Sci., Uppsala, S-750 07, Swed.
SOURCE: Journal - Association of Official Analytical Chemists (1984), 67(3), 503-6
CODEN: JANCA2; ISSN: 0004-5756
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Liquid chromatographic determination of the plant estrogens coumestrol and **isoflavones** in animal feed
AB A simplified procedure is described for the cleanup and detn. of the 5 main plant estrogens in animal feed: coumestrol [479-13-0], **genistein** [446-72-0], biochanin A [491-80-5], **daidzein** [486-66-8], and formononetin [485-72-3]. The estrogens are extd. with

EtOH and purified on a Sep-Pak C18 cartridge. All 5 plant estrogens are sepd. in an isocratic liq. chromatog. system and quantitated by UV and fluorescence responses. The method is sensitive to about 2.5 ppm coumestrol and 10 ppm of the **isoflavones**.

IT Feed analysis
(**phytoestrogens** detn. in, liq. chromatog.)

L3 ANSWER 211 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1984:135976 CAPLUS
DOCUMENT NUMBER: 100:135976
TITLE: Dynamics and distribution of **phytoestrogens** in aerial organs of meadow clover during ontogenesis
AUTHOR(S): Agaev, F. N.; Samorodova-Bianki, G. B.; Mukhina, N. A.
CORPORATE SOURCE: Vses. Inst. Rastenievod. im. Vavilova, Leningrad, 190000, USSR
SOURCE: Fiziologiya i Biokhimiya Kul'turnykh Rastenii (1984), 16(1), 46-52
CODEN: FBKRAT; ISSN: 0532-9310
DOCUMENT TYPE: Journal
LANGUAGE: Russian

TI Dynamics and distribution of **phytoestrogens** in aerial organs of meadow clover during ontogenesis

AB The **isoflavone phytoestrogen** content and compn. of clover organs varied with respect to organ and plant age. Leaves contained primarily biochanin A (BA), formononetin (FN), and **genistein** (GN), stems and flower buds contained BA, FN, and **daidzein** (DN), and flowers contained all 4 compds. The highest FN and BA contents were found during regrowth of medium-early, medium-late, and late clover varieties, whereas early varieties had the highest FN, BA, and GN contents during flowering. Leaves had the highest estrogen contents during regrowth, stems and flower buds during flower bud formation, and flowers at the beginning of flowering. GN and DN were found only in some varieties, and at 2-8-fold lower levels than those of BA and FN. Southern varieties with high estrogen contents were less winter resistant than varieties with a low estrogen content (.ltoreq.350 mg/100 g fresh matter). By selection 8 varieties with low estrogen content were produced.

ST clover organ estrogen growth; **phytoestrogen** clover growth

IT Plant breeding and selection
(of clover with low **phytoestrogen** content)

IT Plant growth and development
(**phytoestrogen** content and distribution in clover during)

IT Clover
(T. pratense, **phytoestrogen** content and distribution in organs of, during plant growth)

L3 ANSWER 212 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1981:3169 CAPLUS
DOCUMENT NUMBER: 94:3169
TITLE: Effect of some factors on **phytoestrogen** levels in red clover
AUTHOR(S): Palfii, F. Yu.; Malik, O. G.; Lun, M. I.; Dlyaboga, O. R.
CORPORATE SOURCE: Nauk.-Dosl. Inst. Zemlerobstva Tvarinnitstva Zakhidnikh Raioniv, Obroshino, USSR
SOURCE: Visnik Sil's'kogospodars'koi Nauki (1980), (9), 35-9
CODEN: VSNAAG; ISSN: 0042-7020
DOCUMENT TYPE: Journal
LANGUAGE: Ukrainian

TI Effect of some factors on **phytoestrogen** levels in red clover

AB The estrogen activity (in diethylstilbestrol equivs.) and the **phytoestrogen** (**isoflavone**) content of red clover depended on growth stage, pptn., and fertilization. The estrogen activity increased with increasing pptn., was high at high pptn. during intensive

growth when 240 kg P/ha was applied and highest during flowering when P 240 and K 2 kg/ha was given. The estrogen activity was least at low pptn. during flower-bud initiation when P 120-240 and K 120 kg/ha was applied. The **isoflavone** content of clover decreased with increasing pptn. At <70 mm rain/mo the activity of the **phytoestrogens** decreased during flower-bud initiation and intensive growth when any fertilizer was applied and during flowering when N 240 and P-K (360:360 kg/ha) were applied. Other fertilizer rates increased the **isoflavone** content of the plants at other than above growing stages. When **genistein** concn. (in relation to total **isoflavones**) in plants increased, resp. decreases in biochanin A and its 4'-Me ester were noted. Generally clover contained more formononetin and biochanin A than **genistein** and **daidzein**, in that order. No relation between the **isoflavone** content and estrogen activity in clover was found.

ST clover estrogen **isoflavone** rain fertilizer

IT Plant growth and development
(estrogen activity and **isoflavone** content of clover during)

IT Soil moisture
(estrogen activity and **isoflavone** content of clover in relation to)

IT Fertilizer experiment
(with macronutrient traits, estrogen activity and **isoflavone** content of clover in)

IT Waters, natural
(rain, estrogen activity and **isoflavone** content of clover in relation to)

IT Clover
(T. rubrum, estrogen activity and **isoflavone** content of, factors affecting)

L3 ANSWER 213 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1980:526770 CAPLUS
DOCUMENT NUMBER: 93:126770
TITLE: Lack of mutagenicity of some **phytoestrogens** in the salmonella/mammalian microsome assay
AUTHOR(S): Bartholomew, Richard M.; Ryan, Dale S.
CORPORATE SOURCE: Dep. Food Sci., Univ. Wisconsin, Madison, WI, USA
SOURCE: Mutation Research (1980), 78(4), 317-21
CODEN: MUREAV; ISSN: 0027-5107
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Lack of mutagenicity of some **phytoestrogens** in the salmonella/mammalian microsome assay

AB Eight **phytoestrogens** were tested for mutagenicity using a variation of the Salmonella/mammalian microsome (or Ames) assay. Zearalenone [17924-92-4] is a mycotoxin produced by a grain contaminant, Fusarium graminearum (Gibberella zeae) and the isomers of zearalanol [26538-44-3] are reduced derivs. of this compd. The remaining compds. are all flavonoids which occur naturally at relatively high concns. in many plants, particularly legumes. Four of these flavonoids (**daidzein** [486-66-8], **genistein** [446-72-0], formononetin [485-72-3], and biochanin-a [491-80-5]) are **isoflavones** and the 5th, coumestrol [479-13-0], is a coumestan. Each compd. was tested at 1-500 .mu.g/plate. The microsomal fraction was obtained from Aroclor 1254-induced rat livers. None of the compds. tested was mutagenic to Salmonella strains TA1538, TA98, or TA100 at any concn.

ST **phytoestrogen** mutation Salmonella

IT Salmonella
(mutation of, from **phytoestrogens**)

IT Mutagens
(**phytoestrogens** as)

L3 ANSWER 214 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1971:496322 CAPLUS
DOCUMENT NUMBER: 75:96322
TITLE: Comparison of plasma **phytoestrogen** levels in sheep and cattle after feeding on fresh clover
AUTHOR(S): Braden, A. W. H.; Thain, R. I.; Shutt, D. A.
CORPORATE SOURCE: Div. Anim. Physiol., CSIRO, Prospect, Australia
SOURCE: Australian Journal of Agricultural Research (1971), 22(4), 663-70
CODEN: AJAEA9; ISSN: 0004-9409
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Comparison of plasma **phytoestrogen** levels in sheep and cattle after feeding on fresh clover

AB The metabolism of formononetin and biochanin A was qual. similar in sheep and cattle, but in cattle formononetin was more rapidly metabolized and the circulating **phytoestrogens** and their metabolites were more efficiently conjugated. Formononetin and its metabolites **daidzein**, equol, and O-demethylangolensin, and biochanin A and **genistein** were identified in the plasma of wethers and heifers fed on red clover or subterranean clover. The ratio of formononetin concn. in the plasma to the total concn. of its 3 metabolites was much higher in wethers than in heifers, and the ratios of the concns. of unconjugated and conjugated **isoflavones** and metabolites were usually higher. On the basis of increase in teat length in wethers, red clover (Trifolium pratense) and the Tallarook cultivar of T. subterraneum had approx. equiv. estrogenicity; Mt. Barker subterranean clover was much less active.

ST plasma **phytoestrogen** sheep cattle; formononetin plasma

IT Clover
(**phytoestrogens** of blood plasma of ruminants in response to)

IT Cattle
Sheep
(**phytoestrogens** of blood plasma, clover effect on)

IT Blood plasma
(**phytoestrogens** of, of ruminants, clover effect on)

L3 ANSWER 215 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1971:1178 CAPLUS
DOCUMENT NUMBER: 74:1178
TITLE: Uterotropic activity and heritability of biochanin A and formononetin in Trifolium medium
AUTHOR(S): Gourley, L. M.; Keim, Wayne F.; Stob, Martin
CORPORATE SOURCE: Dep. Agron., Purdue Agr. Exp. Sta., Lafayette, IN, USA
SOURCE: Crop Science (1970), 10(5), 503-6
CODEN: CRPSAY; ISSN: 0011-183X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 6-clone diallele cross was used for anal. Large quantities of the **isoflavone phytoestrogens** biochanin A (I) and formononetin (II) and small amts. of **genistein** and **daidzein** were found in the hybrid zigzag clover (T. medium): Anal. of variances indicated that the significant genetic variance for the **isoflavones** was primarily explained by general combining ability. The upper leaflets contained about 1 3 more I and II than lower leaflets. The internodes contained about 1 10 as much II as leaflets and only a trace of I.

ST **phytoestrogens** clover; clover **phytoestrogens**; **isoflavones** uterotropic Trifolium; uterotropic **isoflavones** Trifolium; Trifolium uterotropic **isoflavones**

IT Clover
(**isoflavone phytoestrogens** of Trifolium medium, genetics in relation to)

IT Genetics
(**isoflavone phytoestrogens** of clover in relation to)

L3 ANSWER 216 OF 540 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1967:479671 CAPLUS
DOCUMENT NUMBER: 67:79671
TITLE: Paper chromatography of **isoflavone phytoestrogens**
AUTHOR(S): Chury, Jiri
CORPORATE SOURCE: Veterinarmedizinischenfak., Brno, Czech.
SOURCE: Sbornik Vysoke Skoly Zemedelske a Lesnicke v Brne, Rada B (1966), (3), 279-83
CODEN: SVZBAX
DOCUMENT TYPE: Journal
LANGUAGE: German
TI Paper chromatography of **isoflavone phytoestrogens**
AB Paper chromatog. was used to sep. **isoflavone phytoestrogens** from lucerne and clover exts. Formononetin was identified as the main **isoflavone** in red clover. The ext. from these plants contained also biochanin A, **daidzein** and **genistein**. In the ext. of Trifolium repens biochanin A was not found. In these plants formononetin predominated. Lucerne ext. contained 4 **phytoestrogens** but formononetin predominated. The amts. of these substances were less than in clover ext. It appeared that estrogen activity in clover and lucerne was due to formononetin.
ST FORMONONETIN CLOVER; ESTROGENS PLANTS; **PHYTOESTROGENS** CHROMATOG; **ISOFLAVONE PHYTOESTROGENS**; CHROMATOG **PHYTOESTROGENS**; CHURY J
IT Estrogenic hormones
RL: BIOL (Biological study)
(**isoflavone** phyto-, paper chromatog. of)

L3 ANSWER 217 OF 540 MEDLINE on STN
ACCESSION NUMBER: 2003584504 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 14664520
TITLE: **Phytoestrogens** Modulate Binding Response of Estrogen Receptors alpha and beta to the Estrogen Response Element.
AUTHOR: Kostelac Drazen; Rechkemmer Gerhard; Briviba Karlis
CORPORATE SOURCE: Institute of Nutritional Physiology, Federal Research Centre for Nutrition, Karlsruhe, Germany, and Institute for Biofunctionality in Food, Technical University of Munich, Munich, Germany.
SOURCE: Journal of agricultural and food chemistry, (2003 Dec 17) 51 (26) 7632-5.
Journal code: 0374755. ISSN: 0021-8561.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20031216
Last Updated on STN: 20031216
TI **Phytoestrogens** Modulate Binding Response of Estrogen Receptors alpha and beta to the Estrogen Response Element.
AB . . . induces gene activation and is an important step in estrogen-induced biological effects. Here, we investigated the effects of some dietary **phytoestrogens** such as the **isoflavones genistein** and **daidzein**, its metabolite equol, and the coumestane coumestrol on the binding rate of ERalpha and ERbeta to ERE by a nonradioactive. . . to bind to ERE immobilized on the surface of a sensor chip even in the absence of estrogens. 17beta-Estradiol and **phytoestrogens** induced an increase in ER binding to ERE in a concentration-dependent manner. 17beta-Estradiol was a more potent activator of binding than the **phytoestrogens** studied. The concentrations of 17beta-estradiol inducing an increase in the binding response of ERalpha and ERbeta to ERE by 50%. . . the sequence and the

EC(50) were as follows: 17beta-estradiol (0.03 microM) > coumestrol (0.2 microM) > equol (3.5 microM) > **genistein** (15 microM) > **daidzein** (>300 microM) and for ERbeta 17beta-estradiol (0.01 microM) > coumestrol (0.025 microM) > **genistein** (0.03 microM) > **daidzein** (0.35 microM) > equol (0.4 microM). The ratios EC(50)alpha/EC(50)beta were calculated to be for 17beta-estradiol, 3; coumestrol, 8; equol, 8.8; **genistein**, 500; **daidzein** > 850. These ratios indicate that **genistein** and **daidzein** preferentially activate the binding of ERbeta to ERE. The endogenous hormone 17beta-estradiol as well as coumestrol and **daidzein** metabolite equol activate the binding of ERbeta to ERE only slightly more effectively than the binding of ERalpha to ERE. Thus, the effect of **daidzein** can be changed from a specific activator of ERbeta to an activator of both ER isotypes alpha and beta in humans who are able to convert **daidzein** to equol. While the results of the measurements with ERalpha were in line with the binding affinities of compounds tested for ER, there was a distinct difference between our results and the binding affinities of **phytoestrogens** for the ERbeta. This leads to the conclusion that **phytoestrogens** differ not only in their binding affinities for the ER, but also in their potential to increase the rate of. . .

L3 ANSWER 218 OF 540 MEDLINE on STN
ACCESSION NUMBER: 2003559056 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 14638870
TITLE: Flavone and **isoflavone phytoestrogens** are agonists of estrogen-related receptors.
AUTHOR: Suetsugi Masatomo; Su Leila; Karlsberg Kimberly; Yuan Yate-Ching; Chen Shiuan
CORPORATE SOURCE: Department of Surgical Research and Division of Information Sciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010, USA.
CONTRACT NUMBER: CA44735 (NCI)
ES08258 (NIEHS)
SOURCE: Molecular cancer research : MCR, (2003 Nov) 1 (13) 981-91. Journal code: 101150042. ISSN: 1541-7786.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20031126
Last Updated on STN: 20031219